

Support for Trieste

THE International Centre for Theoretical Physics in Trieste was established in the early sixties in order to provide a base to which young competent theoretical physicists from developing countries could go occasionally for intellectual exchanges and advanced research. An extensive report on the centre's activities was given earlier this year in *Nature* of March 22. Much of the early, and indeed continuing success has been due to the manipulations of Abdus Salam, then Pakistan's delegate to the International Atomic Energy Agency (IAEA) and Paolo Budini, professor of physics at the University of Trieste. These two managed to persuade IAEA, the city of Trieste and the Rome government to support a project which encountered a lot of cynicism and disbelief from the scientifically advanced countries. Many felt that helping physicists from technologically backward countries to pursue esoteric research was wasteful of scarce manpower resources. Salam vigorously attacks this point of view by pointing to the alternative open to bright young physicists who discover the exhilaration of research—emigration. 'In a developing country there may be a mere handful of highly skilled academic physicists', he declares, 'They each play a major role in the development of that country's technology, universities and intellectual life. Better far to provide somewhere to recharge their batteries than simply to let them emigrate.'

In the early days the city provided a total of \$1 million and IAEA gave \$55 thousand annually. UNESCO started with \$27 thousand annually and in 1970 moved up to a fuller level of participation. At present the Italian government gives \$350 thousand, UNESCO, the UN Development Project and IAEA each give \$200 thousand.

UNESCO support, although modest at first, is, at least formally, ten years old and UNESCO pursues a policy of regarding its financial aid as no more than seed money to get an institution going. After ten years, according to its protocol, the centre should be able to stand on its own feet and find sources of money elsewhere. This is a sound policy for most establishments that UNESCO supports which can go to a specific national government and ask for money. But it is less sound where an institute is used by 90 different nations, the majority poor.

The official British line, which will lead to the delegation expressing their concern at UNESCO this week, is that after ten years there are other deserving projects which could use the \$200 thousand—itsself a large slice of the total UNESCO basic science budget of \$1.5 million—and so the support should be re-examined. And a 34% growth rate, they say, is out of line with other growth rates in UNESCO. There is no immediate pressure on Trieste, as it is not proposed to cut funding at once; however in the long run it is clear that the British delegation want Trieste to look elsewhere for their money. Other countries do not see it that way, indeed

one or two diplomat-scientists privately express the view that Britain is making herself look foolish by such a gesture at this time. With inflation posing terrible problems for all educational institutions and the rest of the world apparently unready to rock the boat, what causes Britain to take a maverick line?

Partly, no doubt, there is a feeling in the Ministry of Overseas Development (which concerns itself with UNESCO) that the way to help developing countries is through more 'relevant' research. But the prime mover in this affair seems to have been a distinguished scientist himself—Sir Harold Thompson, chairman of the Royal Society UNESCO scientific committee.

It had been clear for some time that Sir Harold intended to urge UNESCO to withdraw from Trieste. His views on the centre (*Nature*, 243, 136; 1973) were that it was successful, but he did express some concern that international laboratories were being backed by 'well organised pressure groups'. Yet it was not until May 1974 that a group of physicists and mathematicians familiar with the centre was convened by the Royal Society to put their point of view to Sir Harold.

The meeting seems to have been unsatisfactory in that there was no real meeting of minds over the issue. The group, pressure group that it doubtless was, left dissatisfied and with the feeling that the nature of the Trieste centre and the problems that it faces had been barely understood. Trieste's mathematical activities—a recent development—had hardly been appreciated.

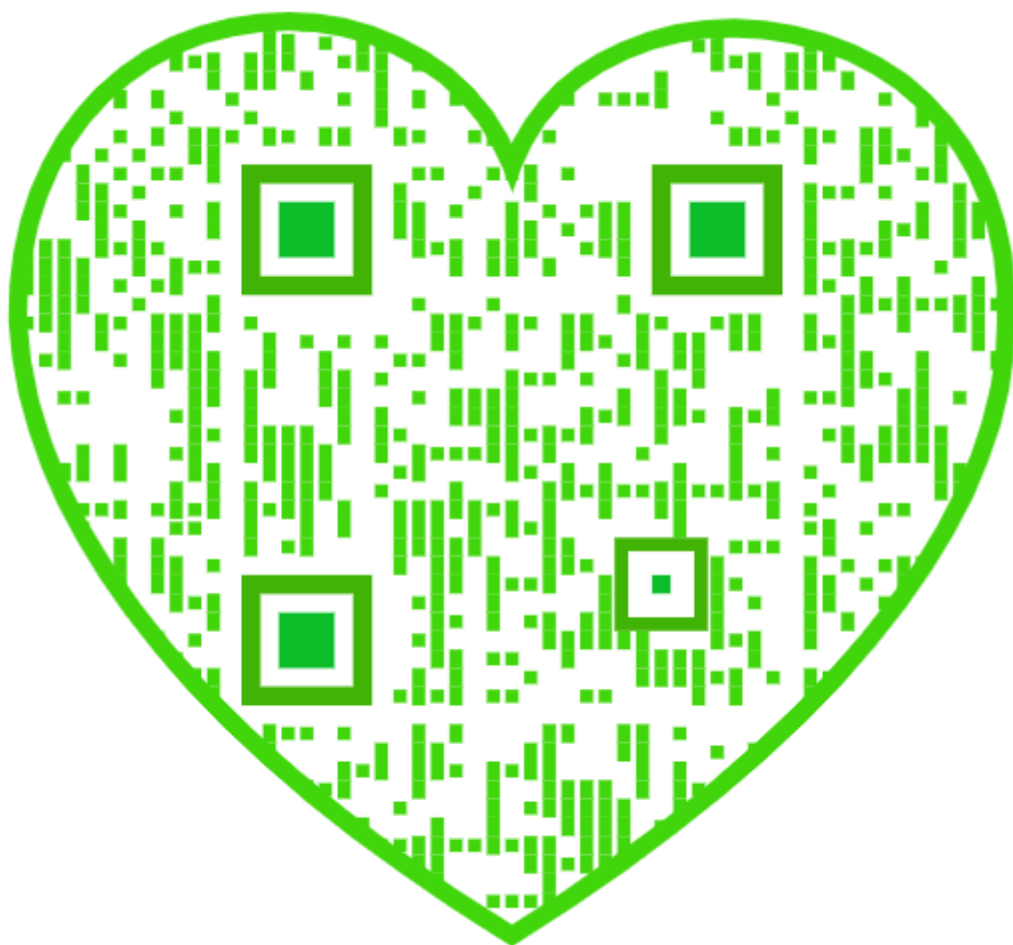
As a result, Britain's official viewpoint cannot really be said to be representative of the views of British physicists and mathematicians and seems as much as anything to have emerged from one man's opinion.

The growth rate of 34% quoted by those who urge re-examination is a mystery. It is a real growth rate, we were assured. And yet UNESCO's contribution has risen only from \$150 thousand to \$200 thousand so far in the seventies, and only inflation is permitted to modify this figure before 1976. Hardly a real growth of 34% and certainly not per annum.

It is, of course, entirely within Sir Harold's rights to press for a strict interpretation of the UNESCO rules if he wishes. But surely when so many others are deeply concerned, an opinion which flies in the face of the advice of fellow scientists is worrying, and a mechanism by which a Royal Society-appointed committee can go to an international meeting with an un-representative view needs examination. And the figures should be right.



A LARGE monumental fountain, ornamented by the celebrated sculptor Carpeaux, has been erected on the Observatoire Place at Paris. It represents Europe, Asia, Africa, and America rotating the globe, which they carry on their heads, and is very effective; but in spite of M. Le Verrier's protestations, they are rotating the globe from east to west, according to the Ptolemean theory.



international news

Monod's answer to the Pasteur's problems

THE financial problems of the Pasteur Institute have long been clear to the French public and to workers at this famous French biological research institute. The Pasteur has been in the red for the past 15 years and has been obliged to conduct many fund-raising campaigns through the press and television, as well as seeking government aid.

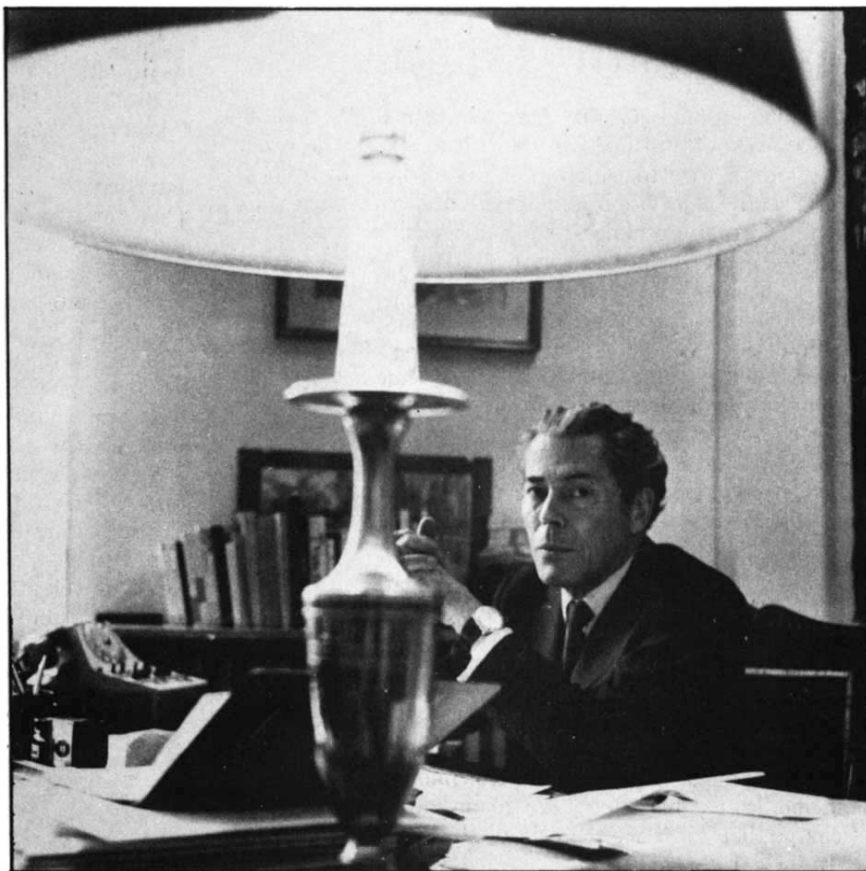
It now seems that this has not been enough to save the situation, which has been further aggravated recently in an unforeseeable way.

At a meeting on October 11, Professor Jacques Monod, director of the institute, announced to the scientific staff that there was a risk that employees and suppliers would not be paid after May 1975. Most of the assets of the Pasteur Institute have already been sold and no more credit can be obtained.

The annual amount spent by the institute will reach about 65 million francs by the end of 1974, of which various government contracts will pay for around a third. Unfortunately the institute cannot count on profits from its new factory for producing vaccines and sera, set up in Normandy in 1973. Although sales are satisfactory, production costs have risen much higher than expected. Profits are thus much lower than predicted and financial equilibrium is several years off.

Added to this gloomy financial situation is the deplorable condition of many of the buildings centred around the Rue Doctor Roux. Only the departments of molecular biology and virology occupy new or renovated buildings. The department of bacteriology is scattered over several buildings, some of which date from the time of Louis Pasteur himself. In some cases even normal security precautions cannot be enforced. In crumbling lecture theatres, students have to write on their knees. The building of a long awaited new immunology department seems to have been adjourned *sine die* for financial and administrative reasons.

Unless a great deal of work is started quickly, Professor Monod fears that a creeping paralysis will overcome work



Monod at the Pasteur: a bold and attractive plan.

at the institute. But in the present parlous state of the finances, even the urgent improvements cannot be started.

To disentangle the institute from this mess, Professor Monod has just presented a bold and attractive plan to the institute's Council of Administration and the public authorities. He wishes to sell all the buildings and land in the Rue Doctor Roux and rebuild the institute at an entirely new site at Garches, 10 kilometres to the south-west of Paris. Because of the enormous rise in land values in the centre of Paris, the sale would raise sufficient funds to enable the institute to build its new laboratories and still leave a profit of 70 million francs. An operation on this scale could, of course, not be realised without government approval. Also, many of the scientists, although admiring Professor Monod's effort and imagination, ask whether the project is realistic in the present uncertain economic climate in France. The fact that the financial forecasts for past years have turned out to be wide of the mark

and that details of the financial situation remain unknown to most of the workers, clearly raise doubts among the researchers about the validity of the new project. The Pasteur Institute is being asked if all possible efforts have been made to find a less grandiose but less risky solution, and it is feared that the financial and technical crisis may precipitate a crisis amongst the staff.

Whatever the final solution turns out to be, it will be a painful one for the institute and those who work there. Research teams may not survive the upheavals of the next few years and some researchers and technicians may well leave the institute.

At the end of November, the government representatives on the Administrative Council will give the government's decision and a final decision will be arrived at on December 19. In the meantime, M. Royer, the President of the Administrative Councils plans to hold a meeting with the staff in the absence of Professor Monod.

FROM THE STAFF OF LA RECHERCHE.

THE recent assurances given by the Soviet government to the United States about the position of Jewish would-be emigrants seems to promise a considerable improvement in the lot of those scientists dismissed from their posts and subsequently subjected to official harassment as a consequence of their application for a visa for Israel. That is, provided a serious attempt is made to implement them! A spokesman for the Medical and Scientific Committee for Soviet Jewry admitted, however, to a "certain amount of scepticism" concerning the announcements. "When we actually see the bargain being implemented . . . , when the activists in Moscow are allowed to go, when Voronel is free from harassment and this seminar allowed to function without interruption, then we shall feel that our work has borne fruit. Not until then. Meanwhile Voronel is once more in hiding and the trial of Dr Shtern is due to begin."

The case of Dr Mikhail Shtern, Director of the Department of Endocrinology at a hospital in Vinnitsa (Ukrainian SSR) does, indeed, seem to justify this cautious attitude. Dr Shtern and his wife applied in September 1973 for visas for Israel. Since then they have been subjected to continuous and systematic harassment, including searches of their apartment, the dismissal of their sons, both scientists, and their sons' wives, from their professional posts, and the interrogation of Dr Shtern himself on a number of charges.

These have changed, over the months, from straightforward dissidence, "causing the hospital to harbour incorrect ideological tendencies" and "accepting bribes from patients", to attempted murder of child patients. This last accusation, on which Dr Shtern currently faces trial, represents a new trend in the pattern of accusation—would be emigrants having been in-

Promises, promises

from Vera Rich, London

dicted so far mainly on relatively minor charges of "hooliganism". To the observer, it seems reminiscent, on the one hand, of the notorious 'Doctors' plot' of the last days of Stalinism and, on the other, of an atavistic re-emergence of the old "ritual murder" charge.

Although the charge is far more serious than any so far brought, the pattern is running true to form. It hardly needs the statements made last September by two relatives of former patients of Dr Shtern, that the investigating authorities have been compelling patients to give false testimony that "the doctor whom they had trusted and respected for 30 years intended and attempted to poison their children", to see in this prosecution the usual harassment of the would-be emigrant; indeed, in May, the Pro-

curator of Vinnitsa admitted as much himself. Nevertheless, the gravity of the charges is evoking considerable concern in the West, and a number of eminent scientists and doctors, including Lord Hunt of Fawley, Professor William Stanley Peart, and Professor Sir Ludwig Guttman have taken part in a campaign of letters, telegrams and telephone calls to the All-Union and Ukrainian Medical Academies, and the Vinnitsa Procurator's Office—so far without any effective reply.

The trial was scheduled for October 30 but enquiries made by Dr Shtern's sons, Viktor and Avgust, to the Procurator's office, elicited only the information that the legal proceedings were "no business" of theirs. The only news forthcoming is that the 56-year-old doctor is in a serious state of health, with internal haemorrhage from a duodenal ulcer and tuberculosis. He is at present being held in an underground room and is to be visited by a medical commission.

It is, of course, possible that the refusal of the Vinnitsa Procurator's Office to reveal any details of the proceedings may be a first stage in the tacit dropping of the charges as part of the new agreement. Even if this should be the case, the manner of the deed, the almost sadistic detailing of the prisoner's medical condition to relatives allowed no access to him, can hardly be seen as a favourable augury for the promised new deal for Soviet Jews.

WITH talk of massive cutbacks in federal spending to help fight inflation, and with the 1976 budget just three months from publication, some members of the scientific community in the United States are getting the jitters. And well they might, for expenditures on science and technology fall almost totally in the so-called controllable category of the federal budget, thus making them prime targets for reduction.

One area which is clearly going to be hard pressed is high energy physics, and the Zero Gradient Synchrotron (ZGS), a proton accelerator located near Chicago, is a likely candidate for extinction. The Office of Management and Budget (OMB), which prepares the Administration's budget request, has already decided that the ZGS should soon be consigned to the technological scrap heap, for earlier this year it asked the Atomic Energy Commission and the National Science Foundation to develop a "plan for shutting down the ZGS accelerator at the earliest possible time".

The rationale for that decision is that since money for high energy physics is unlikely to grow in these

Foot on the brake for accelerators

by Colin Norman, Washington

times of financial stringency, and with the new Fermi National Accelerator soaking up a rapidly growing proportion of funds available for that esoteric science there just is not enough money to operate all the particle accelerators at a reasonable level. The ZGS machine being the least powerful of the high energy accelerators, and also being located close to the new Fermi accelerator, looked like the logical one to shut down.

There is, of course, nothing new in such a trend. To accommodate the growing operating budget for the Fermi machine—which began work about two years ago—the Atomic Energy Commission cut off funds for the Princeton-Pennsylvania accelerator in 1971, axed the Cambridge Electron Accelerator a year later, and is now in the process of converting the Bevatron from a

high energy machine into a heavy ion accelerator.

But, having decided that the ZGS should be the next to go, the office of Management and Budget must have been astonished at the advice it received on how the deed should be done. A special AEC-NSF committee which was set up to develop a plan for shutting down the ZGS, told the budget cutters in an unpublished report last month that it found the ZGS programme to be "vigorous and innovative" and that it could see "no scientific or technical reason to recommend a shutdown at this time". Ask a silly question . . .

The committee pointed out that some features of the ZGS are unique, and that just because it is operated at a relatively low energy does not necessarily mean that it is the most expendable of the high energy accelerators. To confuse the OMB people even further, the committee recommended that operation of the ZGS should be continued "possibly at a more intensive level" for the next four years, and that mid-1979 should be considered the earliest reasonable time to shut the machine down. □

Radical medicine in Beersheba

from Nechemia Meyers

ISRAEL'S fourth medical school, attached to the Ben-Gurion University of the Negev, opened this month in Beersheba with a first-year class of 34 students, two of them Arabs.

Was there any justification for yet another medical school in a country with the world's highest ratio of physicians to potential patients (about three per thousand) and hundreds of students at existing schools, not to speak of the 1,500 Israelis studying medicine in Europe? Many people, particularly those connected with the other three medical schools, think not. But thanks to the missionary zeal of its chief advocate, Professor Moshe Prywes, it is now an established fact.

Prywes, connected for many years with the Hebrew University Medical School in Jerusalem, is determined to develop a very different kind of institution at the Ben-Gurion University, of which he is president.

He wants to use the new school as a means of breaking down the barriers that now exist between academic medical centres on one hand, and neighbourhood clinics on the other. At present, Israel's best qualified doctors, graduates of her own medical schools, cluster around the universities and university-affiliated hospitals, leaving day-to-day medical care to be dispensed mainly by old-timers and new immigrants. And the neighbourhood doctors, being effectively cut off from hospitals and research centres, tend to practice out-of-date, conveyor-belt medicine—which makes them frustrated and their patients dissatisfied.

Prywes aims to ensure that the graduates of his school are different by training them to be patient-oriented from the outset. For example, the schools calls for students to work in clinics and hospitals, under the supervision of special tutors, from their very first year.

Internship will also be handled in a new way. Instead of becoming interns at the end of their studies, the fledgling medicos will spend a full year working in the field, albeit with limited responsibilities, midway through their course.

The Ben-Gurion University Medical School, unlike others in Israel, will play down basic biological research, emphasising instead research linked to the specific health problems of the community. While not in any way denying its importance, the directors of the new school advise would-be students who are primarily interested in research to enrol elsewhere.

This same philosophy is reflected in

the selection of members of faculty, among whom are men who have never written a scientific paper in their lives. In Prywes's opinion, physicians who have proved themselves as lecturers and as doctors in the field are as qualified to be professors as are research-oriented MDs with dozens of papers to their credit.

These unorthodox professors, it is hoped, will set an example for their students. "Hitherto," Prywes has pointed out, "neither the medical student nor the resident saw his teachers working outside the hospital or the research laboratory. Thus talking to students about the importance of community medicine was bound to be a waste of time. They could hardly be convinced of the significance of something their own mentors ignored." □

Stormy weather hits Mexican research

FLOODS and very early frosts have destroyed several years' worth of research at the International Wheat and Maize Improvement Center, the internationally renowned plant breeding institute run by Norman E. Borlaug in Mexico. Frost killed off most of the hybrid wheat and maize being bred at two research stations near Mexico City early in September, and floods associated with hurricane Fifi completely devastated a third station in eastern Mexico three weeks later.

In most cases the crops—which represented the results of two or three years of cross breeding—were destroyed before they had produced seed for planting next year. According to Dr Robert Osler, deputy director of the center, there is no reserve seed from intermediate hybrids so it will be necessary to go right back to the original strains and cross breed them again over the next two or three years to reproduce the destroyed plants.

Osler said that up to 80% of the wheat and the entire maize crop was destroyed at a station at Toluca near Mexico City by frosts which arrived several weeks earlier than usual. The same frosts also wiped out most of the maize at another station on the other side of Mexico City, although much of the wheat crop there survived. Osler fears, however, that because the frost caught the plants at a relatively early stage the seeds may be difficult to germinate.

Flood damage to the third station is more serious. Torrential rain associated with hurricane Fifi—the one which devastated Honduras—caused a river to burst its banks and flood fields at a research station located at Poza Rica to a depth of 3 metres. Osler said that the water took the topsoil off a

large area and flooded silos and machinery sheds.

Osler estimated that it could take at least a year to repair the damage, one problem being that replacing the topsoil could introduce a good deal of variability in soil condition. The Center has applied to the Consultative Group, an international group of donors which supports crop breeding research, for funds to repair the damage. There is also a possibility that a European government may provide at least part of the money. □

Rhodesia rules

"THE University [of Rhodesia] has been exempted by the British Government and others from sanctions." So runs the last sentence of a sheet describing Salisbury, its university and the chair of biochemistry which was being advertised recently. What does this mean in practice? One of our readers enquired of the Foreign Office in London and got the following replies:

Can I convert European currency into Rhodesian currency? Travellers to Rhodesia are normally only permitted to take £25 in sterling notes.

What personal goods can I take? Household and personal effects, but not cars.

Can I order and receive scientific research equipment? Export to Rhodesia is prohibited except by licence issued only for humanitarian reasons (religious, educational or medical). Each application is considered separately; present policy is to give "sympathetic consideration to applications from the University of Rhodesia".

Can I advertise in the United Kingdom for technical and administrative personnel? Only under licence (the same criteria apply as above). Present policy is to issue licences for academic but not for other posts.

Can I seek research grants in the United Kingdom? Nothing prevents you, but the transfer of funds is subject to strict exchange control rules—each case is considered on its merits.

Can I change Rhodesian currency back into other currencies? It cannot be exchanged in the United Kingdom. "I cannot say what facilities are available in Rhodesia."

He's not going.

We called the Foreign Office for clarification of some items and they draw attention to two points. First, nothing can be taken for granted in dealing with the United Kingdom from Rhodesia. There are no automatic exemptions and everything has to be reviewed. Second, there is no British representative in Rhodesia, so none of the customary consular facilities either of a routine or an emergency nature are available. □

THE abrupt departure last week of Dr John Sawhill as head of the Federal Energy Administration (FEA) came as no surprise to those who have been keeping track of the jockeying for power and political backstabbing which has characterised energy planning in the United States for the past 18 months. Sawhill, after all, had been in the job for six months, which is longer than anybody else managed to survive, and although he was generally regarded as an effective administrator who won praise from Congress and consumer organisations, he has trodden on a good many important toes recently.

His resignation—which can be described as forced rather than voluntary—paved the way for the appointment of Andrew E. Gibson as the new administrator of the FEA. Gibson's background is not in energy policy but in maritime affairs—he is a former Maritime Administrator and Assistant Secretary of Commerce—but that is not surprising since relevant experience has rarely been a criterion for appointment to a top energy job in either the Nixon or Ford Administrations.

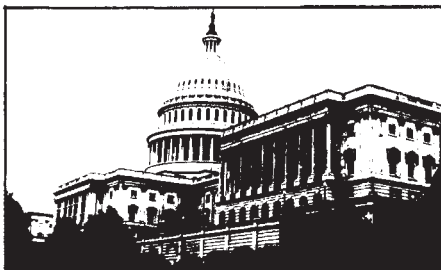
To put last week's events in context, it is instructive to examine some of the bureaucratic musical chairs which have been taking place in energy policy since April last year. At that time John Ehrlichman was nominally in charge of energy planning in his capacity as Chairman of the Domestic Council, but when he became otherwise engaged Nixon created a National Energy Office to advise on policy.

That arrangement lasted only a few weeks before Nixon scrapped it and formed an Energy Policy Office in the White House to coordinate energy programmes. He persuaded John Love to resign as Governor of Colorado to come to Washington as the new energy czar and DiBona was retained as a consultant.

Love's reign lasted only until October, when Nixon announced yet another reorganisation which entailed scrapping the Energy Policy Office and creating a Federal Energy Office, a more powerful body with a large staff and a mandate to develop energy policies and to carry out day-to-day operations. William Simon, a spectacularly successful Wall Street investor who was then Deputy Secretary of the Treasury, was named as head of the office and Love, embittered by his shoddy treatment, returned to Colorado where he is now practising law. DiBona also departed to become executive director of the petroleum industry's chief lobbying office. The Federal Energy Office's name was later changed to the Federal Energy Administration when Congress sanc-

tioned its appearance on the Washington landscape.

In April this year, Simon became Secretary of the Treasury following the departure of George Shultz from the government, and Sawhill, who was Simon's deputy, was promoted to head the agency. But it is important to note that Simon retained a lever on energy planning because he hung on to the chairmanship of an inter-agency coordinating committee which consisted



Washington seen

by Colin Norman

of top officials of government agencies carrying out energy programmes.

All that was changed by President Ford, however, who last month created an Energy Resources Council as the overall manager of the federal government's energy policies and named his old Congressional friend Rogers C. B. Morton, the Secretary of the Interior, as its chairman. That put Morton firmly in charge (he also retains his job as Secretary of the Interior) and higher in the energy bureaucracy than Sawhill and Simon. Since the two are said to be at loggerheads on some issues and since Sawhill recently annoyed White House officials by openly advocating that a 30% tax be slapped on gasoline, Morton forced his resignation.

In view of that chronicle of events, it is small wonder that a coherent energy policy has yet to emerge from the Administration.

● On the same day that Sawhill's resignation was announced, another shuffle of top officials concerned with scientific matters took place in Washington. Robert Seamans, President of the National Academy of Engineering and former Secretary of the Air Force, was named as head of the new Energy Research and Development Administration (ERDA), Dixy Lee Ray, Chairman of the Atomic Energy Commission (AEC), has been appointed Assistant Secretary of State for Oceans and International Environmental and Scientific Affairs, and William A. Anders, a former astronaut, has been named as head of the new Nuclear Regulatory Commission (NRC). The shuffle is the result of Congressional approval of a

bill to dissolve the AEC and replace it with the ERDA and the NRC.

Seamans, an aeronautical engineer, will take charge of virtually all of the federal government's energy research and development programme, and his appointment has so far been generally welcomed. He faces a tough job, however, in getting the ERDA off the ground since bureaucratic jealousies are sure to arise when programmes are shifted from existing federal agencies into the new organisation. And, since the ERDA will be built around the laboratories of the Atomic Energy Commission, he will be faced with the difficult task of striking a balance between nuclear and non-nuclear research.

The appointment of Anders has, however, drawn mixed reaction. As head of the NRC, Anders will be placed in charge of regulating nuclear power plants and ensuring that they meet safety criteria. He has been a Commissioner of the AEC for some 15 months, where his chief responsibility has been to oversee the breeder reactor programme. It is that factor which has drawn some sniping, since the breeder reactor has become one of the chief targets of nuclear critics.

Finally, Dixy Lee Ray has been appointed to fill a newly created post in the State Department. The result of a reorganisation plan dictated by Congress, the position is designed to elevate scientific affairs in the department by bringing them together.

● Two reports published recently by the National Science Foundation (NSF) catalogue a trend of declining federal budgets for science and technology and of declining employment of scientists and engineers by the federal government.

First, in spite of modest increases in spending on science and technology in the past two years, the NSF reports that when inflation is taken into account, the science budget for this year is "below that of any year during the 1965-75 decade." In short, expenditures on research and development have climbed from \$16,800 million in 1973 to \$17,700 million in the financial year 1973-74 and \$19,600 million this year. But, in terms of 1967 dollars, the purchasing power of the science budget has declined from about \$17,500 million in 1967 to about \$13,000 million, the NSF suggests.

Another set of statistics shows that, for only the second time in 20 years, the number of scientists and engineers employed by the government dropped last year. In October 1972, there were 166,700 scientists and engineers on the federal payroll, but a year later the number had declined to 161,500.

India parcels out its energy problems

from Narender Sehgal

RECENT changes in the central council of ministers, following a cabinet reshuffle, have resulted in the creation of a separate Ministry for Energy, to be headed by a minister of state (slightly lower in status than a full fledged cabinet minister). This, however, does not mean that all subjects relating to energy have been brought under one roof. Far from it; as of now, only 'power' (from the erstwhile Irrigation and Power Ministry) and 'coal' (from 'mines' which until now had been under the charge of the Ministry of Steel and Mines) have been assigned to the new ministry.

Fears are already being voiced about the administrative problems that may arise from the change. For example, much of the hydroelectric power produced (37% of the total generated in India by all means) is tied to irrigation projects, hence their association in a single ministry. (Irrigation will now form part of the Ministry of Agriculture.)

Oil, which accounts for 50% of India's entire consumption of energy derived from commercial sources, remains, as before, part of Petroleum and Chemicals under the charge of a cabinet minister.

The diversity of coal users (railways and the steel and fertiliser industries, for example) presents an additional problem of administration and co-ordination. It remains to be seen if it is possible to retain all matters concerning coal within the ambit of the newly created Ministry of Energy.

Then there is atomic energy which remains under the direct control of the Prime Minister. In this area of increasing importance, Shrimati Gandhi has been in the past assisted by Shri K. C. Pant who will continue in this role even after he has taken charge of the new Ministry of Energy.

On the basis of the past record of co-operation and coordination between various ministries, it is not possible to feel overly enthusiastic about this new attempt to solve India's grave and growing energy problems.

● India is known to have uranium in only small amounts. Known deposits, at present rates of production and consumption (with due allowance for planned future uses) would last no longer than some 30 years. On the other hand, China is understood to possess uranium in fair abundance in relation to her immediate and long term needs. One would think, then, that China could not possibly have any use for Indian uranium.

No so. Men arrested by the Bihar police recently, with varying quantities of uranium in their possession, have revealed that the stolen metal was meant to be smuggled to China through Nepal and that another 100 kilograms or so was awaiting disposal at various places in Bihar. An editor of the Hindi daily at Patna, engaged in investigating the smuggling operations and possessing some related documents, was reportedly murdered and relevant documents stolen by the apparently well organised gang of uranium smugglers, just before the newspaper was about to publish the story.

The uranium in question was stolen from the Jadugoda mines near Jamshedpur in Bihar. While investigations and more arrests are continuing, the view is gaining ground that the motivation behind the Chinese interest in Indian uranium may have to do with her desire to weaken or slow India's atomic energy programme. □

Weather watch down under

by John Gribbin

THE Commonwealth Meteorology Research Centre, which was set up in 1969 with a five year lease of life, has recently been reconstituted and provided with an extension for a further five years, under the auspices of the Australian government. Now the Australian Numerical Meteorology Research Centre (ANMRC), this Melbourne based operation is playing a key role in studies of the problems of meteorological research in the southern hemisphere.

These problems are formidable indeed. Because of the great proportion of ocean in the south the observational network is far from complete; in addition, the distribution of what land there is means that population in the southern hemisphere is concentrated in the tropical and near tropical regions, north of 40°S. And far less is known about the behaviour of weather systems at these latitudes than at extra-tropical latitudes.

During its first five years of existence under the "Commonwealth" label, the centre was closely allied with the Global Atmospheric Research Programme (GARP), and this spirit seems likely to continue. In July of this year, when the centre was renamed, the Australian government affirmed support for the next five years, with research objectives defined in the following terms:

"The work of the Centre consists of studies of the behaviour of the earth's atmosphere, with emphasis on general

circulation, directed towards improvement in the accuracy and timescale of weather forecasting, and towards improvement in understanding the distribution and variations in climate on the earth."

The work at the ANMRC is, as its name suggests, strongly orientated towards computer studies, with facilities available on the IBM 360/65 at the World Meteorological Centre in Melbourne (this centre is the southern counterpart of the two WMCs in Washington and Moscow, the three forming part of the World Weather Watch). With the aid of modern satellite photography, many of the handicaps resulting from the shortage of observing stations in the south can be overcome; satellite and other data are combined in mathematical models of atmospheric flow which have already produced reasonable forecasts for one to four days ahead. Improvements are likely to come on two fronts: through introducing more representations of physical processes into the model (better 'skill'); and through refining the numerical methods used (better 'efficiency').

In global terms, the Australian work is particularly interesting because it offers an alternative mathematical approach to some of the problems which beset meteorologists attempting to produce global models. So far, such models have encountered difficulty because of problems near the poles, and although progress is being made there is a need for different approaches to resolve the difficulties. The ANMRC has been working with the so-called 'spectral method', which involves representing the atmospheric variables by a truncated series of spherical harmonics, and with a combination of spectral techniques and grid techniques, which they refer to as a 'semi-spectral' or Fourier model.

In a draft version of part of the 1974 Annual Report of the ANMRC (as yet unpublished) it is stated that "a significant proportion of the operational products [*sic*] now issued by the National Meteorological Analysis Centre of the Australian Bureau of Meteorology are numerically derived", although "manual intervention" is still an important part of the forecasting process. The draft goes on to say that the Fourier model is now also proving useful in basic atmospheric research, and is helping to provide an insight into the dynamics of the stratosphere and mesosphere; in future years, as the research programme matures, the ANMRC sees a greater emphasis on such problems as the influence of volcanic debris and of anomalies in sea surface temperatures on climatic change. □

The language of space

from Angela Croome

Lt.-Colonel Alexei Leonov, commander designate of the Soyuz crew for next summer's Apollo-Soyuz link-up (the ASTP) announced a nice compromise on the question of space communications during the joint presentation on ASTP progress at the Amsterdam International Astronautical Congress. Last year it was not known what the language of space would be, said Leonov; it had now been decided that on the mission the Russians would speak English and the Americans Russian. In an emergency each team would revert to its own language. It had not however been fixed who would take overall command in a crisis.

The 'book' of operational proce-

dures had been written and practised in the four joint training sessions. Emergency procedures had yet to be worked out and there was further polishing of the language to be done; this had proved much more of a problem than originally envisaged (though judging from Leonov's competent performance, his English was no longer a headache). Still to be fully sorted out was the ticklish question of the interrelation of the two control centres. There seemed no clear indication that an American team of controllers would be sitting in at Baikonur next July.

Both the Russians and the Americans assured questioners that there was nothing to choose between the two sides in terms of input to the engineering and other design and operational concepts. Nonetheless behind the

scenes American space planners were saying the flow of information was all one way and in the space medicine field in particular there was a great scarcity of adequate Soviet data. How had the Soviet programme eradicated early problems with motion sickness? Skylab astronauts, groggy and sick during their first week in the orbital workshop, would dearly like to know.

Medallurgy

We are agog to see whether our hint (*Nature*, 246, 113; 1973) that the Royal Society's policy for the distribution of medals was just a trifle inward-looking has fallen on fertile ground. It's medals-time again next week and several Fellows have told us they will try and persuade Council to look further afield this year.

correspondence

Radioactivity salted away

SIR—Referring to the article "Fruits of a Faustian bargain" (*Nature*, 251, 274; 1974) it appears that the United States propose tests in 1980 for the storing of radioactive waste so as to stop dumping it—as do the UK, USSR and other nations—in the ocean. The German authorities seem to be ahead of them, as for several years now, low and moderately radioactive waste has been stored in a salt mine close to Hannover by the Gesellschaft für Strahlen-und Umweltforschung owned by the Federal German Republic. And there is still space for thousands of barrels for decades to come. It may be taken for granted that no earthquake will occur to wreak havoc—for millions of years none has happened there, so the only thing to fear is mankind itself by trying to prove its efficiency by discovering a new and final way of self destruction.

HANS K. KOENBER

*Laser & Electro-optik,
D-8000 München*

Plasmid moratorium

SIR—The appeal by a committee of eminent biomedical scientists for a voluntary moratorium on an area of scientific research which may create unpredictable hazards to human health (*Nature*, 250, 175; 1974) reminded me of a talk which Leo Szilard gave at a writers' club in Moscow in December 1960 which I attended, and where to my knowledge the question of a moratorium was raised for the first time. This story was later told by Szilard

and published¹.

"A year ago last December I was in Moscow to attend the sixth Pugwash conference. And while I was there I was invited to talk to a writers' club about molecular biology, about my work. One of these writers said, 'Now what practical consequences does this have?' So I said, 'As far as I can see it has no practical utility whatever—but of course if you had asked me that about nuclear physics in the 1930s I would have told you the same thing.' And then the Russian said, 'Well in that case, wouldn't it be better if you stopped right now?'"

Yours faithfully,

GERTRUD WEISS SZILARD

La Jolla,

California 92037

¹ *Thinking Ahead with Leo Szilard*, in *Int. Sci. Tech.*, 33–38 (1962).

Sitting Bull?

SIR—I was startled to read (*Nature*, 251, xviii; 1974) that Colorado State University is encouraging 'ethnic minorities and women' to apply for the position of Chairperson in its Statistics Department. I reject as highly improbable the implicit assumption that ethnic minorities necessarily consist entirely of males. Nevertheless, I wonder whether the aim is to encourage collective applications resulting in large-scale migrations of American Indians (that is deviations from their normal distribution). Does this mean that sophisticated statistical tests (excluding Student's *t*, of course) will then be used to select individual chairpeople

from large samples? And, even if this analysis is at variance with the real object of the exercise, won't the unsuccessful applicants sioux?

Yours faithfully,

F. A. SMITH

*Department of Botany,
University of Adelaide,
Adelaide, South Australia 5001*

ILL reactor?

SIR,—Although apposite and allusive alliterations are always amusing and appropriate as acronyms, Institut Laue-Langevin still satisfies many of us. It recalls that the construction of this long awaited reactor is owed to a Franco-German agreement, whilst with 1,000 visitors and 300 completed experiments in the last year, the Institut is already known as ILL the world over.

I believe we should 'let ILL alone'.

Yours faithfully,

W. M. LOMER

*Institut Laue-Langevin,
Grenoble, France*

Erratum

SIR In my news note (*Nature*, August 9) credit for the fallopian tube transplant experiments in animals should have gone to Dr B. M. Cohen of the University of Cape Town Medical School, not to his close colleague Dr M. Katz, who has drawn my attention to the error. I apologise to both gentlemen.

IAN RIDPATH

*35 Oakwood Gardens,
Ilford, Essex*

news and views

How to have your fibrous cake and eat it

FIBRE reinforced plastic composites combine the high strength and modulus of strong fibres with a high work of fracture (toughness). Two letters in this issue describe methods of improving the work of fracture without significantly reducing the strength.

A high work of fracture allows one to use a material secure in the knowledge that in a structure it will show signs of failure long before it actually breaks. It might be thought that the combination of brittle fibres with a brittle resin would produce a brittle composite. The toughness of these materials is the result of energy-absorbing processes at the weak fibre-resin interface during fracture. The most important of these processes is probably the work of pulling out broken fibres after the crack tip has passed, leaving a whiskery fracture surface. This work decreases as the interfacial bond strength is increased because the crack then cuts more cleanly through the fibres leaving shorter pull-outs. On the other hand, to weaken the bond strength also weakens the composite.

A new approach demonstrated by Atkins (page 116) is to produce alternating strong and weak bonded regions on the fibres. The strong zones maintain the composite strength, the weak regions allow lengths of fibres to debond, break and pull out so toughening the material. He uses boron fibre-epoxy resin composites with bands of silicone grease or polyurethane varnish on the fibres to produce weak bonding while, he claims, the uncoated regions will be strongly bonded. The varnish-coated composite shows a remarkable increase in toughness at coating levels above 50% and the strength is maintained up to 90% coating. For the silicone grease the toughness increase is much more modest. The results all fit Atkins's analyses for strength and toughness. The increase in toughness with varnish coating

is from 50 kJ m^{-2} with no coating to 200 kJ m^{-2} at 90% coating with no strength loss. Conventional surface treatments would only be expected to produce variations of a few per cent about the lower value without also changing the strength.

What is not clear yet is whether the varnish and grease both do act as weak coatings relative to uncoated fibres. The difference in the results for the two coatings suggests that the full explanation is more complicated. The system is obviously of commercial interest but also could be scientifically productive since our knowledge of this interface (like that of most interfaces between different phases or materials) is very limited. Carbon fibre reinforced plastics may be expected to show similar improvements but it is interesting to note that the silane treatments used on glass fibres are known often to form beads on the glass surface rather than smooth coatings.

A second approach to toughening is described by Gordon and Jeronimidis (page 116), who have considered the arrangement of cellulose fibrillae in the cell walls of timber. Gordon has remarked before that trees seem to have opted for a high work of fracture rather than maximum strength or modulus. Reasoning that this might be associated with the helical arrangement of the fibrils around the cell wall, Gordon and Jeronimidis now show that under stress the helical tube collapses and the structure undergoes extensive deformation before breaking. They report that model composites constructed on the same basis show very high work of fracture values (400 kJ m^{-2}). They report no strength or modulus measurements but theoretically the decrease in modulus should be small and presumably strength will behave similarly.

Many other approaches to improving the low toughness of boron and carbon fibre reinforced composites are also being studied. But there is a long way to go before materials of the complexity of bone, ligament or timber are developed and before the material is designed concurrently with the structure in which it will be used.

P. D. CALVERT

Micrometeoroid density from lunar craters

THE Solar System not only contains planets, moons, asteroids, comets, meteorites and meteoroids but also a plethora of smaller particles known as micrometeoroids. These are so small that they do not burn out when they enter the Earth's atmosphere but are gently retarded by momentum transfer to the air molecules and slowly float to the ground. All particles with masses less than 10^{-6} g fall into this category, the Earth's surface collecting 3,000 tonnes of this material each year, enough to form a layer of dust 1 cm deep during the Earth's lifetime. The physical properties of these particles can only be measured indirectly. Experiments flown on satellites and space probes measure mass, velocity and sometimes orbit. Rockets can be used to collect the particles in the atmosphere but only pick up the micrometeoroids after they have been retarded by the atmosphere and contaminated by meteoric dust ablated from larger meteoroids. Studies of zodiacal light lead to values for the particles' dielectric constant and shape.

A new and exciting way of studying the properties of micrometeoroids is by investigating the surfaces of the

small glassy spherules found in the samples of lunar soil returned to Earth by the Apollo and Luna space programmes. These spherules are formed like raindrops during the solidification of the molten ejecta blown out from the lunar surface during impact cratering and volcanic events. They then lie on the surface, exposed to space and to the micrometeoroid influx. As the Moon has no retarding atmosphere the micrometeoroids strike the spherules at velocities between 5 and 20 km s^{-1} and produce small craters.

Spherules have around 5,000 microcraters (diameters a few μm) per square centimetre of their exposed surface, the numbers of craters increasing with decreasing crater diameter. A typical microcrater is shown in Fig. 1.

Smith, Adams and Khan have concluded—in a communication on page 101 of this issue of *Nature*—that by comparing the ratio of crater depth to diameter in microcraters on the surface of lunar spherules with this ratio for laboratory-produced impact craters in glasses they can estimate the densities of micrometeoroids, the percent-

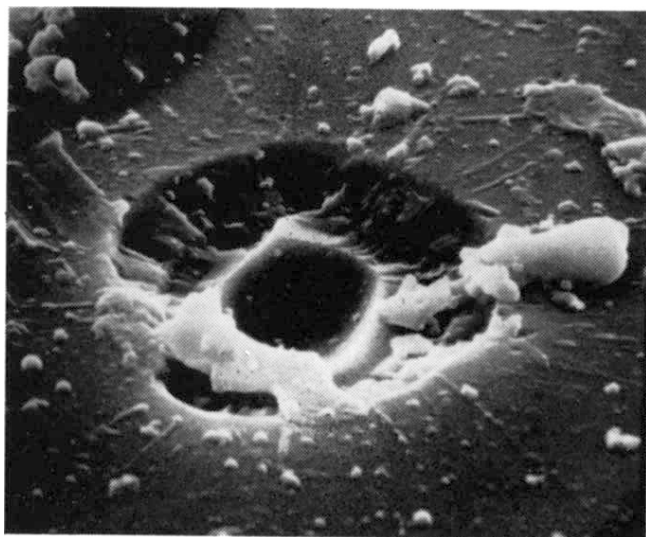


Fig. 1 A 13 μm impact crater on a lunar glass spherule. The spall zone has removed the raised rim and contains radial and concentric fracture patterns (NASA S-71-45362; courtesy of Friedrich Hörz).

ages of the micrometeoroid influx having differing densities and the way this percentage varies with particle mass. They find that the microcraters fall into three distinct groups having depth to diameter ratios of 0.94 (deep craters), 0.50 and 0.33 (shallow craters). By measuring craters produced by impacting hypervelocity ($\sim 3 \text{ km s}^{-1}$) iron, aluminium and polystyrene pellets on to soda lime glass, tektite glass and quartz the authors conclude that the ratio of crater depth to diameter decreases when the projectile density decreases and that the three different ratio values found for lunar microcraters are produced by iron micrometeoroids (density 7.9 g cm^{-3}) stones and carbonaceous chondrite micrometeoroids (density 2.7 g cm^{-3}) and low density (1.2 g cm^{-3}) particles, possibly spongy micrometeoroids or even ice crystals. The percentages of high, medium and low density particles in the $\sim 2 \mu\text{m}$ diameter range are 40, 20 and 40% respectively. Measurements were also made in the laboratory of the ratio between the crater diameter and the diameter of the incident particle. The authors find that iron particles produce craters of twice their own diameter, the spongy micrometeoroids producing craters having the same diameter as the projectile—so the mass of the projectile responsible for a crater can now be calculated easily. Considering particles with diameters greater than $2 \mu\text{m}$ the incident mass is found to be made up of 20% iron micrometeoroids, 10% stony micrometeoroids and 70% spongy micrometeoroids. So spongy micrometeoroids which for a long time have been thought to be the predominant type in the radio meteor range of the mass spectrum (that is, $>10^{-6} \text{ g}$), also predominate down to about 10^{-10} g , a result which indicates that some differentiation process must have occurred during the production of these small particles.

Many events have been suggested to explain the origin of micrometeoroids—among them being debris from asteroidal collisions, condensations in the outer solar corona, accretion and condensation of interstellar material, remnants of the primordial dust cloud that produced the planets and debris from decaying comets. This new result should help to narrow the choice. It can be compared with observations of meteorites, the large bodies ($>10^3 \text{ g}$) which penetrate the atmosphere and can be subsequently collected and analysed. Here irons make up 6% by number, stones and stony irons 94%. So these two types of particle of very different size have entirely different relative proportions of irons and stones.

Smith, Adams and Khan also find that the nature of

micrometeoritic material depends strongly on the region of the mass spectrum being considered, the proportion of iron particles increasing as particle size decreases. The region with particle diameters between 1 and $2 \mu\text{m}$ (mass $\sim 4 \times 10^{-12} \text{ g}$) is found to be a region of transition, larger particles having predominantly low densities, smaller particles having predominantly high densities. This is another very important result because it could be the first definite observation of the effect of radiation pressure on the micrometeoroid dust cloud. In the Solar System the force due to radiation pressure opposes that due to solar gravitational attraction and the two forces cancel out for spherical particles at 1 AU when the product of the particle diameter and density equals $1.2 \times 10^{-4} \text{ g cm}^{-2}$. For particles of densities of 7 and 1 g cm^{-3} the limiting diameters are 0.2 and $1 \mu\text{m}$ respectively, smaller particles being quickly blown out of the solar system. $1 \mu\text{m}$ should then be the 'cut off' size for low density particles and this is exactly what Smith, Adams and Khan have found. For smaller particles to have stable orbits they must have high densities. Assuming that 7.9 g cm^{-3} is the highest particle density an extrapolation of the authors' results indicates that no particles of mass less than 10^{-14} g can come within 1 AU of the Sun and have a stable orbit. So the increase in particle numbers with decreasing mass should halt at 10^{-14} g . Smaller particles than this should be very rare, the few present having been produced in the recent past and moving in hyperbolic orbits as they escape from the Solar System. But this prediction is difficult to check because 10^{-14} g is right at the limit of detection for satellite, space probe and lunar cratering experiments.

DAVID W. HUGHES

The linear differentiation of chromosomes

FROM the first description of an induced differential staining of plant chromosomes following cold treatment (Darlington and La Cour, *J. Genet.*, **40**, 185; 1940) twenty-eight years were to elapse before Caspersson and his colleagues (*Expl Cell Res.*, **49**, 219; 1968) showed that chromosomes treated with quinacrine mustard displayed a series of lateral fluorescent bands. This technique became known as Q banding and was quickly followed in other laboratories by the development of the C, G, R, G₁, T, N and Cd banding and the DNA/RNA hybridisation *in situ* techniques. These techniques have had great value in the recognition of individual chromosomes and the analysis of translocations and other rearrangements—since extended to all hybrids, especially man-rodent hybrids. They have introduced to human cytogenetics refinements comparable with the contribution of the salivary gland chromosomes to *Drosophila* cytogenetics. In addition, they have stimulated speculation about the biophysical and biochemical organisation of chromosomes which this article attempts to review.

Discovery of the causes of C, G and Q banding should illuminate the long-standing distinction between constitutive heterochromatin and euchromatin, now found to be repetitive and unique DNA sequences respectively. Most methods involve treatment of acetic/alcohol fixed chromosome spreads in solutions of varying pH, temperature, and ionic strength followed by staining with Giemsa or fluorescent dyes, though the age and quality of preparation are found to be of great importance. The principles are often more important than the actual recipe and the successful outcome is so variable that the methods have been compared to Chinese cooking (*Mammalian Chromosome News Letter*, **15**, No. 2; 1974).

Histone distribution, AT/GC base ratio and denaturation

Maintaining genetic diversity

AMONG the many recommendations of the United Nations Conference on the Human Environment (Stockholm, 1972) which are beginning to bear fruit are those on the conservation of genetic resources. Registration of bacterial culture collections and seed banks is already under way. Now an *Index of Living Plant Collections in the British Isles* has been compiled by Dr J. T. Williams (Bentham-Moxon Trust, Royal Botanic Gardens, Kew, 1974). In his introduction to the index Professor Heslop-Harrison says that "the compilation of the present index was not inspired directly by the UN recommendations [having been started before the Stockholm conference] but its intent is wholly consonant with them".

This preliminary index lists institutions with collections of higher plants and collections actively being used in research. It is being distributed worldwide by the International Union for Conservation of Nature and Natural Resources; possibly also by the Food and Agriculture Organisation of the United Nations, as a step towards the ultimate aim of an international register of living plant collections.

of DNA were each proposed as possible causes of Q, G and C banding patterns. Histones however were shown to be partially extracted by fixation and even when they were completely removed by HCl post-treatment G banding was not affected (Comings and Avelino, *Expl Cell Res.*, **86**, 202; 1974). Similarly, we have observed (Ockey, *Nobel Symp.*, **23**, 344; 1972) that removal of histone labelled with tritiated precursors is uncorrelated either negatively or positively with G or C banding. Since proteolytic enzymes alone induce G banding and fixation does not seriously denature DNA, differential denaturation was also ruled out as the main cause of Q and G banding. But C banding requires alkali treatment which does induce denaturation with a subsequent rapid renaturation of the highly repetitive DNA sequences in the SSC stage of treatment. Other factors are however involved. Comings and his colleagues (*Expl Cell Res.*, **77**, 469; 1973) and Pathak and Arrighi (*Cytogenet. Cell Genet.*, **12**, 414; 1973) both showed that DNA is lost preferentially from the non-C band regions; this may therefore explain their subsequent Giemsa differentiation but by quantitative autoradiography we have observed (unpublished) that when older ³H-thymidine labelled preparations are used C bands are visible in many chromosomes that show equal loss of DNA from C and non-C band regions.

The C-banding technique is related to the earlier technique of DNA/RNA hybridisation *in situ* (Pardue and Gall, *Science*, **168**, 1356; 1970) using RNA transcribed from satellite DNA. Both involve the denaturation of DNA and both are supposed to locate constitutive heterochromatin yet there may still be some discrepancies between them. The human Y chromosome has always shown up as constitutive heterochromatin in C banding but until now had given no indication of repetitive DNA by the hybridisation method. Recently, however, Evans and his

colleagues (*Nature*, **251**, 346; 1974) showed that the Y contained repetitive sequences of all the four AT-rich satellites so far fractionated from human DNA. Constitutive heterochromatin was originally regarded as being AT-rich, presumably because satellite DNA was AT-rich but this has been found not to be the rule. Other results have indicated that constitutive heterochromatin regions may react differently to different techniques. Some C-banding sites are non-fluorescent with quinacrine as in the human karyotype, but the Y chromosome, which also contains C band repetitive sequences, is the most fluorescent chromosome of the complement. Hoechst 33258, a more recent fluorescent dye, stains the AT-rich C bands in the mouse but not Q bands while in *Microtus* both typical Q and C bands are stained (Hilwig and Gropp, *Expl Cell Res.*, **75**, 122; 1972). Facultative heterochromatin (that of the inactive human X which is represented by the late replicating sex-chromatin body in interphase) behaves like the active X with all known banding techniques, whereas most of the other forms of late replicating heterochromatin can be differentiated by Q and G banding.

It has been proposed that because guanine quenches fluorescence, Q bands must be AT-rich, but this has proved a false trail. Recent work, both with synthetic DNA polymers and with AT-rich satellite DNAs would suggest that the important considerations for quinacrine fluorescence were the sequential arrangement of base pairs, particularly the periodicity of guanine residues which show localised quenching of AT-rich regions, and the availability of binding sites (Selander and de la Chapelle, *Nature new Biol.*, **245**, 240; 1973; Weisblum, *Nature*, **246**, 150; 1973). The recent synthetic polymer studies with Hoechst 33258 by Weisblum and Haenssler (*Chromosoma*, **46**, 255; 1974) show that fluorescence of this compound is enhanced by both

AT- and GC-rich DNA, enhancement being greater however with AT-rich DNA.

Gottesfeld and his colleagues (*Biochemistry*, **13**, 2937; 1974) have carried these studies a stage further with a detailed biophysical study on separated euchromatin and heterochromatin, and found that quinacrine fluorescence was quenched more with euchromatin than with heterochromatin. The DNA extracted from these fractions however showed similar AT/GC base ratios and a similar fluorescence. They suggest from circular dichroism spectroscopy studies that heterochromatin DNA probably exists in the B configuration while euchromatin shows the C form. It is interesting to note in this connection that the conformation of AT-rich DNA in bacteria varies very much in its configuration with relative humidity and over a narrow range of ionic strength of NaCl (Bram and Tougaard, *Nature new Biol.*, **239**, 128; 1972), both of which are critical factors in banding.

The incorporation of base analogues into DNA also affects the staining behaviour of chromosomes. Zakharov and his colleagues (*Chromosoma*, **44**, 343; 1974) have found a considerable reduction in chromosome spiralisation at metaphase in regions which incorporate bromodeoxyuridine (BrdU) and BrdC during the latter period of the S phase when heterochromatin is replicating. No such differential spiralisation was observed when the analogue was incorporated into the early replicating euchromatin, implying a difference in conformation of the DNA between the two forms of chromatin as suggested by Gottesfeld and his group.

Latt (*Science*, **185**, 74; 1974) showed that BrdU, incorporated semiconservatively over two cell cycles, resulted in one of the chromatids showing strong fluorescence (monofilial incorporation), the other (bifilial incorporation) weak fluorescence using the dye Hoechst 33258. Latt explained this difference as a quenching in the bifilial chromatid with less or no quenching in the monofilial BrdU chromatid. This technique provides a valuable tool in analysis of sister-chromatid exchanges (Kato, *Nature*, **251**, 70; 1974). Perry and Wolff (*Nature*, **251**, 156; 1974) were able to obtain a permanent differential staining of chromatids following SSC treatment after BrdU incorporation and subsequent Giemsa staining. The bifilial chromatid was less intensely stained and less condensed than its sister. These results they suggest are probably due to a difference in protein binding to BrdU-containing DNA. They also obtained differential fluorescence with acridine orange in the two chromatids.

Bobrow and Gross (*Nature*, **251**, 77;

1974) have found that in mouse-human hybrid cells the contrasting G_{11} banding behaviour of the parental chromosomes is retained in the hybrid. This will prove not only a valuable asset in karyotype analysis of hybrid cells but also indicates that whatever characteristic of the DNA is responsible for its G_{11} banding response persists even in a hybrid nucleoplasm. Others have approached this problem by studying the structure of the Giemsa molecule involved in staining. Sumner and Evans (*Expl Cell Res.*, **81**, 236; 1973) suggest that the structures of the Giemsa and quinacrine molecules are such that they are able to bridge longitudinally separated sites on the chromosome which have been brought into close proximity by folding of the DNA, and that the spatial arrangement of these sites is influenced by the non-histone proteins. The various banding techniques would then affect the cohesion or the biochemical nature of the non-histone binding. Studies using stains whose chemical specificity is known indicate the possibility of a differential distribution of -SH and -SS- groups in the bands (Sumner, *Expl Cell Res.*, **83**, 438; 1974) or that free NH_2 groups are the binding sites for banding with fluorescent dyes like dansyl chloride (Utakoji and Matsukuma, *Expl. Cell. Res.*, **87**, 111; 1974).

R, G_{11} , T, N and C_a banding may all prove equally relevant to an analysis of the biochemistry and biophysics of chromosomes, but their contribution to date is at a much earlier stage. Progress in understanding the longer established techniques has been slow, partly because few biochemists worked with the cytologists who had made the great technical advances. The many current attempts to explain banding reflect our lack of knowledge of how 1 cm of DNA can be packed with protein into a 1 mm transverse band. But the challenge has now been accepted by the biochemists and the results should soon be fruitful.

C. H. OCKEY

A. J. BATEMAN

Retiring Crab and gamma-ray bursts

from Andrew Fabian and James Pringle

AN informal symposium held at the Institute of Astronomy, Cambridge on October 18 brought together observers and theorists to discuss two separate topics—the Crab Nebula and cosmic γ -ray bursts. Renewed interest is being shown in the Crab Nebula, the remnant of the 1054 AD supernova, because a series of occultations of the nebula by the Moon has just begun. These give experimenters a further opportunity to measure its size and structure. Ten

years ago the Naval Research Laboratory group observed the rate at which the 1–10 keV X-ray flux decreased as the Moon covered the nebula and were thus able to deduce a size of 110 ± 25 arc minutes. Now it should be possible to obtain more accurate results, and at a variety of wavelengths.

Professor Walter Lewin (Massachusetts Institute of Technology) gave a report of his August 13 balloon observations made in northern Canada. The observation was made in the 20–50 keV range and, thanks to the accuracy of the occultation prediction, both the disappearance and reappearance of the nebula were observed. The initial indications are that the emitting region is asymmetrical (approximately 30 by 60 arc seconds with the long axis running NE–SW) and is displaced slightly to the NW of the central pulsar. There seems to be no evidence for a change of size at different photon energies.

Dr Peter Davison (University College, London) discussed two occultations viewed from the Copernicus satellite. The occultation paths, predicted by Dr Leslie Morrison (Royal Greenwich Observatory), are sweeping further south each month, and Copernicus was able to pass through the occultation shadow twice on October 7. In the MIT occultation, the difference in position angle of the Moon's limb on disappearance and reappearance was about 104° , giving two almost orthogonal scans, whereas for Copernicus the difference was 135° , preventing an easy study of asymmetry. The four measurements, this time in the 2.5–7.5 keV band, indicate a size of about 60–70 arc seconds. There is no evidence for the 2.5–7.5 keV emission being centred away from the pulsar position.

At least two more balloon flights and two rocket flights are to be launched and it is hoped that these, with their larger detecting areas, will be able to find details in the structure of the nebula and possibly an unpulsed flux from the pulsar itself.

Dr Jim Felten (Universities of Arizona and Cambridge) and Dr Brian Burn (Universities of Sydney and Cambridge) discussed the theoretical implications of occultation data. It is generally expected that the size of the nebula should decrease at higher photon energies. This is because the length of time for which an electron radiates by the synchrotron process at a given frequency varies inversely with that frequency. Thus if all the emitting electrons are accelerated in the neighbourhood of the central pulsar, only the less energetic ones, radiating at lower frequencies, can retain their energy long enough to stray far into the nebula: some theories predict the X-ray size to be 15 arc s, clearly at variance with the observations. The distribution of X-ray

emission across the nebula should also help to indicate the size of the region in which electron acceleration takes place. To complete the morning, Dr Jacob Shaham (Hebrew University, Jerusalem and University of Cambridge) discussed the possibility of steady X-ray emission from the pulsar itself produced by the release of elastic strain energy created by the slowing down of the pulsar's spin rate.

The afternoon session was devoted to a discussion of cosmic γ -ray bursts. Dr Rodney Hillier (University of Bristol) gave a brief resume of the observations so far and Dr Arthur Bewick (Imperial College, London) reported on a possible detection of another event, made from a balloon-borne detector on September 27. The original discovery of the bursts was made by the Vela satellites each of which contains a small omnidirectional γ -ray detector for the purpose of detecting γ rays from nuclear explosions on Earth. The relative times at which each satellite is triggered can then be used to compute the position of the γ -ray source. Serendipity again came to the aid of science when it was found that the positions of the sources of some bursts of γ rays were not compatible with a terrestrial origin. Other satellites soon verified this conclusion. The total energy received in each burst is about 10^{-4} erg cm^{-2} and the bulk of the energy is in photons of 150 keV, although the exact shape of the spectrum is uncertain. Each burst lasts for a second or so and is often followed by another burst about 5 seconds later. Structure is evident in the data on shorter time scales as well.

The talks on observations were followed by a discussion by Professor Martin Rees (University of Cambridge) of the theoretical interpretation of these observations. Because of the relative lack of data, theorists have yielded to temptation and have had a field day constructing models to produce cosmic γ -ray bursts. There is now almost one theory for each of the twenty or so bursts seen. The power needed to produce each burst is $10^{33} d^2$ ergs, where d is the distance to the source in parsecs. Since there is as yet no clear evidence for anisotropy of the directions from which the bursts originate, it is usually assumed that their origin is either extragalactic or close by in our own Galaxy. Ideas for extragalactic sources include the early stages of radio outbursts in quasars and the outgoing shock wave produced by the explosion of a supernova. Most exponents of the theories involving nearby sources favour neutron stars to produce the energy needed and the short timescale: other suggestions involve scaled up solar flares, possibly from highly magnetic stars, relativistic interstellar grains

and exploding mini black holes.

Old neutron stars are thought to be quite common in the Galaxy and there are two basic means of extracting the required energy from them. First, intermittent accretion either of matter from a companion star or of comet-like bodies could explain the observed bursts. Second, starquakes can release a large amount of energy in a millisecond or so, which can be converted to γ rays either by exciting particles in the star's magnetosphere or, possibly, by dissipation of seismic waves at the stellar surface. In the latter case, it was suggested that observation of nuclear lines from surface nuclei is a possibility. Future observations of these fascinating events should provide a testing ground for this plethora of theories, and should in any case (one hopes) reduce the theory-to-event ratio.

Vertebrate palaeontology and morphology

from Barry Cox

MUCH current work in vertebrate palaeontology proceeds hand in hand with continuing investigations into the life and growth of living vertebrates, to the benefit of workers in both fields. This is the reason for the continuing

success of the annual symposia on Vertebrate Palaeontology and Comparative Anatomy, the 22nd of which was held at the University of Manchester on September 24–26.

The Devonian Gogo Formation of Australia continues to produce a wealth of information, not only because it provides a new early fish fauna, but also because the specimens can be completely freed of matrix to produce undistorted three-dimensional skulls. For example, the Gogo lungfish skulls shown by R. S. Miles (British Museum, Natural History) show clearly the presence of a supraotic cavity similar to that which in living lungfish and amphibians contains an extensive endolymphatic system. Similarly, the Gogo actinopterygian skulls shown by B. G. Gardiner (Queen Elizabeth College, London) provide new evidence that the myodome evolved within that group.

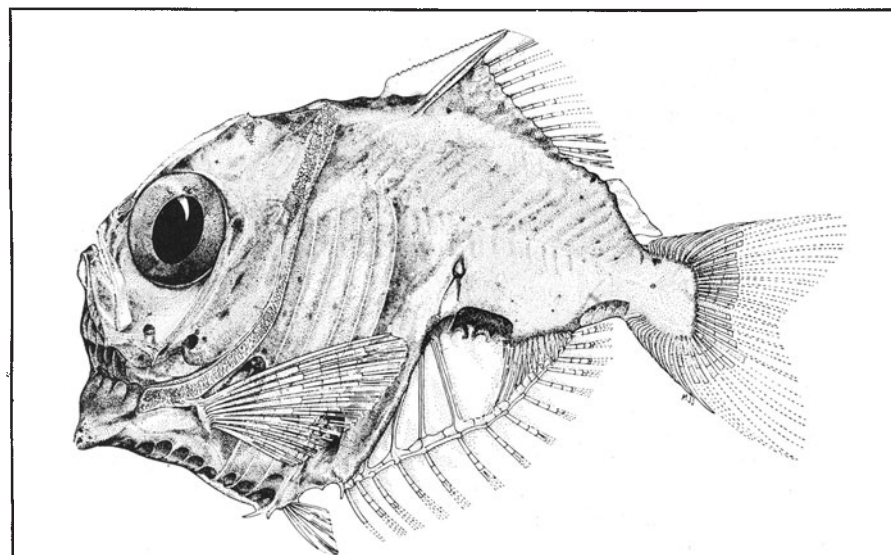
Better understanding of actinopterygian evolution, together with newer methods of phyletic investigation, are bringing more and more order to the taxonomy of the group. What had previously seemed to be taxonomically significant characters are now seen to be primitive, superficial or misinterpreted. C. Patterson (British Museum, Natural History) showed how such misunderstandings had led to the ichthyodectiform fish being placed in a

variety of other taxa, whereas they are now seen to be an independent early teleost lineage. He also pointed out that, in the case of fossil representatives of living groups, it is difficult to use a classificatory scheme to express both their phyletic relationship and their degree of morphological similarity. Patterson suggested that these difficulties could be averted by regarding each monophyletic lineage (irrespective of its rank) as a 'plesion'. These plesions would then merely be listed in a sequence, the order of which would indicate the relative morphological similarity of each to the living sister group.

New material was also described by P. Wellnhofer (Bavarian State Museum, Munich). The fifth specimen of *Archaeopteryx*, though the smallest and apparently juvenile, has the most complete skull; this shows features suggesting that it was kinetic. The accepted view that it is impossible to distinguish convergent evolution in bird osteology was found by C. J. O. Harrison (British Museum, Natural History, Tring) to be unfounded—somewhat to his own surprise.

Two well known morphological/taxonomic features received attention. The function of the hooked fifth metatarsal of lizards was explained by P. L. Robinson (University College, London). Because their proximal tarsals are functionally united with the tibia and fibula, a lever-arm for the gastrocnemius muscle cannot be formed from the calcaneum (as in mammals), but is instead provided by a tubercle on the fifth metatarsal. This fifth digit can also be rotated outwards to provide lateral stability for the foot. A new approach to the old puzzle of the differing composition of the anterior wall of the braincase in Monotremes and in other mammals was suggested by R. Presley (University College, Cardiff), who showed embryological evidence that in the latter this area may be of composite origin, including elements of both epipterygoid and prootic.

Several contributions involved biogeographical data. P. J. Miller (University of Bristol) suggested that the existence of freshwater derivatives of the marine gobies in such river systems as the Po and Arno might have been because the partial drying up, and resulting hypersalinity, of the Mediterranean during the Miocene put a premium on such evolutionary escape strategies. G. L. Underwood (City of London Polytechnic) explained how analysis of various features of the genera of natricine snakes correlated well with their distribution, spreading out from Asia through the islands of the south-west Pacific. C. B. Cox (King's College, London) showed that it was possible to define a series of



THE marine hatchetfishes of the family Sternoptychidae are well known, if mainly for their rather bizarre body form and the generous development of light organs. Three genera are recognised, containing the 25 or so known species, of which *Sternoptyx diaphana* (illustrated) is one of the most widely distributed. Despite several studies of their biology and life style the relationships of the hatchetfishes were not well understood until a recent study by Stanley H. Weitzman (*Bulletin of the American Museum of Natural History*, 153 (3), 331–473; 1974) clarified their placement considerably. By detailed osteological study of several species in each hatchetfish genus as well as of their nearest relatives, Weitzman demonstrates that they are closest to the gonostomatids. With that family, they form one of the major groups of the stomiatoid fishes. Weitzman's rearrangement of the hatchetfishes and their allies is the first to be based on osteological study of adequate material and represents a major advance in the classification of these fishes.

different patterns of faunal regions during the Mesozoic, and that these correlated well with current ideas of the varying patterns of sea barriers due partly to continental drift and partly to marine transgressions.

An elegant synthesis of electromyographic recording and of X-ray ciné film was used by K. Hiiemae (Guy's Hospital Medical School) to analyse the interrelationships between jaw movement and muscle contraction in the opossum.

When particles hit nuclei

from D. J. Miller

How are excited states transmitted through nuclei? For some years it has been clear that we do not know the answer to this question. Surprising results were seen first in coherent production processes, where a beam particle is excited into a low-mass cluster of fast particles, without exciting or breaking the nuclear target. It seems that the fast cluster as a whole has just the same chance of interacting again, on its way out of the nucleus where it is produced, as would a single particle. This applies to clusters of two, three or five fast particles, produced by beams of gamma rays, mesons or nucleons on nuclear targets from deuterium to uranium.

Recent results from the Fermilab (Fermi National Accelerator Laboratory) near Chicago have extended our knowledge of these effects, though there is still no clear explanation. A topical meeting on high energy collisions involving nuclei was held in September at the International Centre for Theoretical Physics in Trieste. New results were reported on a whole range of related topics, including data on the multiplicity of secondary particles in particle-nucleus collisions. Nuclear emulsion workers have shown that the multiplicity of fast charged tracks produced in 200 GeV proton-nucleus collisions is only a modest factor larger than the multiplicity in proton-proton collisions at the same energy. For the mixture of nuclei in photographic emulsion, including a significant amount of bromine and silver, the factor is 1.7, and it has this value from about 70 GeV all the way up to 400 GeV. Busza's group at the Massachusetts Institute of Technology, with a simple Cerenkov counter experiment at Fermilab, has shown that the multiplicity of fast charged particles increases slowly with the atomic mass number A of the nuclide in the target, though the multiplicity in the very forward direction, within 3.5° of the beam direction, is independent of A . These experiments are 'inclusive'; they study

all final states, including the vast majority where the target nucleus has been broken up, in contrast to the unbroken nucleus involved in coherent production.

Tentative theoretical explanations by Gottfried (*Phys. Rev. Lett.*, **32**, 957; 1974), Goldhaber (*Phys. Rev. Lett.*, **33**, 47; 1974) and others have stressed the importance of the 'time dilation' effect of special relativity in understanding the passage of an excited lump of matter through a nucleus. At 200 GeV it takes about 10^{-23} seconds for a particle to get out of a nucleus. But it may take ten times as long for a fast cluster of strongly interacting particles to get out of range of their mutual interaction. The coherent-production data show that the fast cluster behaves like one particle while it is still within the mutual interaction range. The data on inclusive multiplicities seem to be saying the same thing but with the added inference that the decay properties of the cluster are not modified by multiple collisions in nuclear matter. The results so far are fascinating, but there is scope for many more detailed measurements to be made. These could give a new source of evidence on the basic mechanisms of particle scattering, and on the structure of the particles.

Changing views of mantle viscosity

from Peter J. Smith

THE question of the Earth's internal viscosity first became prominent in connection with vertical movements of the crust, in particular with the process of isostatic readjustment. More recently, however, the problem has assumed a much greater significance because of the important part that mantle flow processes are thought to play in the large-scale horizontal motions of continental drift and seafloor spreading. The need to find a mechanism for such motions revived the popularity of the concept of the Earth as rigid lithosphere, viscous asthenosphere, rather more viscous mesosphere and fluid core (as opposed to the equally valid, but apparently less appropriate, division into crust, mantle and core). This emphasis on viscosity then led to much closer examination of the flow properties of the asthenosphere (particularly) and mesosphere, although there is still wide disagreement over the numerical values of viscosity.

In the early studies of isostatic recovery carried out by Haskell (*Am. J. Sci.*, **33**, 22; 1937), Vening Meinesz (*Proc. Kon. Ned. Akad. Wetensch.*, **40**, 654; 1937) and others with reference to the uplift of Fennoscandia, it was assumed that the mantle flows as a

Newtonian fluid and has a uniform viscosity. This work led to the conclusion that, for an infinitely deep medium, the relaxation time (the time taken for the deviation from isostatic equilibrium to decrease to $1/e$ of its initial value) is proportional to viscosity but inversely proportional to the linear dimension of the removed load. Unfortunately, when Crittenden (*J. geophys. Res.*, **68**, 5517; 1963) came to analyse the uplift of Lake Bonneville, he found that the linear dimension differed from that of Fennoscandia by a factor of about 10 but that the relaxation times for the two areas were comparable. By this time, Takeuchi (*J. geophys. Res.*, **68**, 2357; 1963) had already offered a possible explanation of the discrepancy in his suggestion that flow may be concentrated in a thin layer in the upper mantle, basing his argument on the prior conclusion by Jeffreys (*Geophys. J.*, **8**, 196; 1952) that such layered flow would involve a quite different relationship between relaxation time and linear dimension. But this was not entirely satisfactory insofar as the isostatic processes in Lake Bonneville and Fennoscandia were only reconciled by the *ad hoc* assumption that the flow layers beneath the two areas differ in thickness.

As a result, McConnell (*J. geophys. Res.*, **73**, 7089; 1968) decided that the assumption of uniform viscosity must be discarded, and proposed instead a mantle model comprising layers of different viscosity, generally increasing with depth. In the mean time, Gordon (*J. geophys. Res.*, **70**, 2413; 1965) had obtained a broadly similar variation of viscosity with depth by considering flow in the mantle to be due to diffusion creep (Herring-Nabarro creep). Both models led to lower mantle viscosities comparable to the 10^{26} poise obtained by Macdonald (*Space Sci. Rev.*, **2**, 273; 1963) from his analysis of the response of the Earth's shape to the decrease in rotational velocity. Moreover, neither model gave any cause to suppose that the assumption of Newtonian flow need be rejected; on the contrary, in Gordon's model, Newtonian flow was apparently required by the fact that for diffusion creep, stress is proportional to strain (at least for small strains).

But this consistency and agreement was soon questioned by Weertman (*Rev. Geophys. Space Phys.*, **8**, 145; 1970) who concluded that whereas diffusion creep dominates at very low stresses (lower than about 10^{-2} bar), at higher stresses dislocation motion becomes important. Since dislocation motion involves a non-linear stress-strain relationship, the effect of Weertman's conclusion was to overthrow the idea of Newtonian flow in the mantle. His analysis also revealed

a much slower variation of viscosity with depth than that derived from diffusion creep. Specifically, lower mantle viscosity was no higher than about 10^{23} poise, or several orders of magnitude lower than that given by Macdonald. This was not too significant in itself, for Dicke (*J. geophys. Res.*, **74**, 5895; 1969) had recently obtained a lower mantle viscosity of 10^{22} poise from an analysis of the equatorial bulge and Goldreich and Toomre (*J. geophys. Res.*, **74**, 2555; 1969) had suggested a value of 10^{22} – 10^{24} poise to explain polar wandering.

But as Brennen (*J. geophys. Res.*, **79**, 3993; 1974) now points out, the more important problem is that the Weertman curve is apparently inconsistent with the original isostatic recovery data upon which McConnell's model was based. To discover whether the theoretical model of Weertman may be reconciled with the observational isostatic data, Brennen has thus carried out a hydrodynamic analysis of isostatic recovery flow in the mantle. He concludes, first, that the rapid increase in viscosity with depth demonstrated by McConnell and others is simply a spurious effect resulting from the incorrect assumption of Newtonian flow. The real increase of viscosity is much slower and in general agreement with that predicted by Weertman; certainly the strain rates are higher than Weertman's critical value for the onset of dislocation motion. The best fit between the data and the model is then given by a mantle viscosity which increases with depth as $\exp(5 \times 10^{-4}z)$, where z is the depth in kilometres. In short, not only may observation and theory be reconciled, the reconciliation may be taken as evidence that the theory is substantially correct—that is, that mantle flow is non-Newtonian and arises largely from dislocation motion.

Glassy alloys

from Robert W. Cahn

SINCE times prehistoric, metallurgists have quenched iron or steel in water to harden them. Nowadays they know that the hardness is linked to the presence of a metastable crystal form, containing more carbon in solution than iron should at ambient temperature. This crystal form, martensite, has attracted immense research attention, because the genesis, hardness and breakdown of martensite are all absorbingly interesting scientific problems.

It is surprising, then, that it was not until 1960 that anyone reflected seriously upon the possibility of improving on the fastest rate of quenching then available: dropping a sliver of steel into a bucket of water might cool it at a measly rate of perhaps $1,000^\circ\text{C}$

per second. It was always a fair guess that the faster one can quench an alloy, the less chance it has to convert to the equilibrium structure and the more likely it is to be fixed in some abnormal and potentially intriguing crystal structure. Yet no one seriously went into the matter until Pol Duwez at California Institute of Technology, in 1960, recognised that the only effective way to speed up cooling rates substantially was to start from the liquid state. He conceived the idea of blasting a small molten drop of alloy by means of a gaseous shock wave against a sloping piece of copper: the technique soon acquired, over the opposition of its fastidious inventor, the onomatopoeic designation 'splat-cooling'. The drop is atomised and then 'splatted' out into a thin liquid layer, which gives up its latent heat to the copper in a very short space of time.

We now know that splat-cooling and related techniques can generate cooling rates from 10^6 to as much as 10^9 degrees C per second. A complete new metallurgy has been created: highly supersaturated solid solutions, a whole zoo of new metastable crystal structures, and glassy alloys have all been developed. Several hundred research papers have recently been reviewed by Jones (*Rep. Prog. Phys.*, **36**, 1425; 1973): the work covers not only structures but also anomalous mechanical, electrical and magnetic properties.

Of late, there has been a sudden burst of research on the least understood of the new metallurgical species, glassy alloys. The interest stemmed primarily from the work of David Turnbull at Harvard. Turnbull is renowned for his studies on crystallisation of molten metals, in particular his classic studies on the freezing of molten microspheres. It was a natural transition for him to examine the conditions for the congealing of liquid alloys into a glassy state, uncrystallised. He adopted one of Duwez's early alloys, Pd-Si, which could readily be liquid-quenched to a glassy state, and studied its crystallisation on reheating (for example, Chen and Turnbull, *Acta Met.*, **17**, 1021; 1969). Turnbull's ideas then inspired a metallurgist, John Gilman, to more systematic studies. It had already been recognised that alloys of transition metals with metalloids were the most sluggish to crystallise and therefore aptest to form glasses. Gilman became research manager at Allied Chemical Corporation at Princeton and instituted a systematic programme of study. His colleagues, including several of Turnbull's former collaborators, examined a range of alloys based on nickel or iron or mixtures of both, with substantial additions of carbon, boron, phosphorus, silicon and aluminium. They standardised on the 'roller-

quenching' technique: a fine stream of molten alloy impinges on a pair of rollers, very rapidly rotating in contact, and a narrow continuous glassy ribbon emerges. These glasses typically have a glass transition temperature, T_g , in the range of 650–720 K, and a crystallisation temperature, T_c , some tens of degrees higher. Polk and Chen (*J. Non-Cryst. Solids*, **15**, 165; 1974) have recently reported on the characteristics of some of these alloys. One generalisation which emerges is that the stablest glasses—that is, those with the largest value of $(T_c - T_g)$ —are those which contain the lowest metalloid content consistent with glass formation. The metalloid solute seems to exert its crystallisation-inhibiting effect by a combination of simple jamming of metal atoms (because the small metalloids fill up the voids between the metal atoms) and strong bonding between solvent and solute, which inhibits displacive rearrangements. Typical compositions include $\text{Fe}_{75}\text{P}_{15}\text{C}_6\text{Al}_4$ and $\text{Fe}_{38.5}\text{Ni}_{38.5}\text{P}_{18}\text{B}_2\text{Al}_3$.

The glassy ribbons are extremely strong, with ultimate tensile strengths typically exceeding 200,000 pounds per square inch (Americans are incurably addicted to these venerable units). Several of these alloys, notably nickel-based alloys such as Ni-P-B-C-Al, when the roller-quenching rate was high enough, combined very high strength with measurable ductility (Chen and Polk, *J. Non-Cryst. Solids*, **15**, 174; 1974). This is a unique combination: normally very high strength goes with extreme brittleness, as with fine silicon or carbon fibres. The manufacturers expect to apply their ribbons to reinforcing functions, for instance in car tyres.

The physical mechanism underlying the limited ductility of the alloy glasses is also strikingly novel. Pampillo and Reimschuessel (*J. Mater. Sci.*, **9**, 718; 1974) have recently shown that the ribbons deform by a process akin to glide in a metallic crystal. Thin layers of alloy, inclined at 45° to the tensile axis, behave as if they were liquid and the two halves of the ribbon slide over each other. This is probably due to the incipient destruction of short-range (glassy) order and the consequential weakening of resistance to glide. Fracture initiates at several points and the cracks spread along an already defined glide plane, and eventually the cracks collide and the crystal tears apart, showing on the fracture surfaces a pattern of veins which mark the loci along which the cracks collided.

A great deal of work is now in progress on the properties, especially the mechanical behaviour, of these alloy glasses and a number of papers is expected soon in several journals, including the three cited in this survey.

articles

Flux and composition of micrometeoroids in the diameter range 1–10 μm

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A comparative morphological study of microcraters observed on glassy lunar spherules, and of those created on glassy materials in the laboratory, indicates strongly that 'irons' are the major component of the total flux of micron-sized micrometeoroids. This could be because of the differential influence of solar radiation pressure on 'stones' and 'irons'.

WE have compared the natural microcraters which occur on glassy lunar spherules with the craters which are produced on these spherules, and on tektite and soda lime glass and quartz crystals, by the impact of hypervelocity solid microparticles from an electrostatic particle accelerator. A scanning electron microscope and an optical microscope were used to measure the depth and diameter of the craters.

Morphology of craters

We studied a total of 71 craters on 81 light brown, glass spherules (diameters 300–350 μm) from the Apollo 15 fines of less than 1 mm diameter obtained from Spur crater (station 7) near Hadley Rille (sample No. 15301–84). More than 90% of the craters were closely circular in form (indicating near normal incidence) with diameters in the range 1–8 μm and were identified by following the criterion suggested by Hartung *et al.*¹. Laboratory simulation experiments have shown that at expected impact velocities (several km s^{-1}) crater diameters are about 1–3 times larger than the dimensions of the impacting projectile. Therefore, the craters which we have studied should have been formed in micrometeoroid (particles in the micron size regime) impacts.

The crater diameter distribution indicates that the smallest craters are most abundant with a gradual reduction in occurrence with increasing size. A most intriguing result is obtained when the ratio of depth, d , to diameter, D , ($d/D = \chi$) of the craters is considered. A histogram of the number of craters ϕ within a given range of χ (Fig. 1a) indicates that the craters divide into at least two distinct groups. The variation of ϕ with χ does not imply a relationship between ϕ and d or D , as there is no correlation between χ and d , or χ and D . Group I, with a mean $\chi (= \bar{\chi}) = 0.94$, Fig. 1a, is distinctly separated from the second major group which itself may consist of two groups, one centred around $\chi = 0.50$ (Group II) and another centred around $\chi = 0.33$ (Group III) (Fig. 1a). There is some support

for considering the low χ grouping as two separate groups: when the data is represented as a plot of d against D (Fig. 2a), three groupings can be recognised, associated with three lines

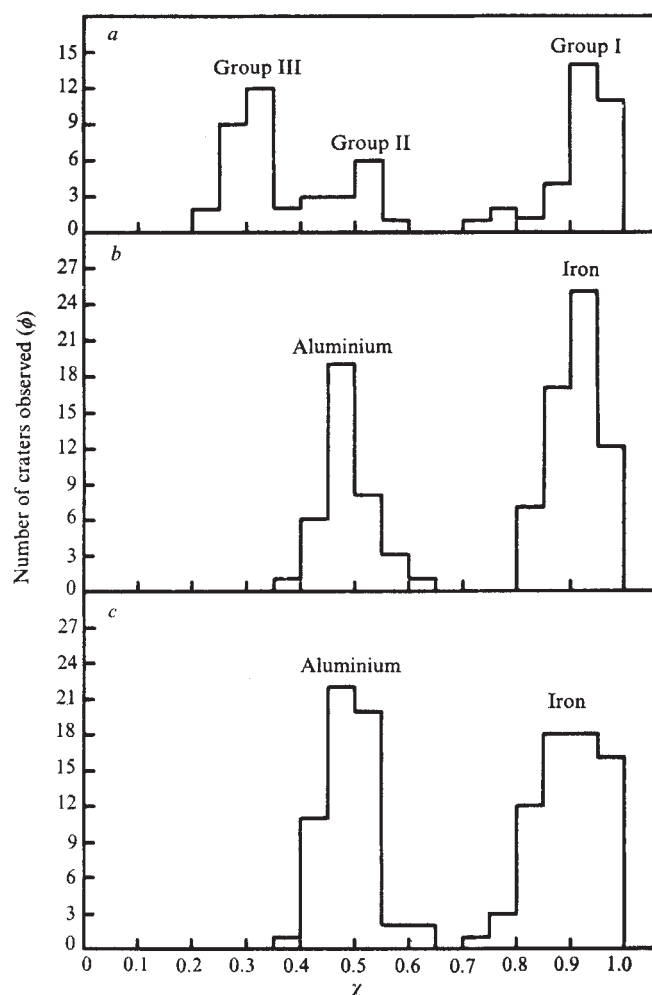


Fig. 1 Histograms of number of craters observed (ϕ) as a function of the crater depth to diameter ratio (χ) for: *a*, natural craters on glassy lunar spherules, showing Groups I, II and III; *b*, craters produced by the impact of aluminium projectiles on tektite glass and iron projectiles on glassy lunar spherules; *c*, craters produced by the impact of aluminium and iron projectiles on soda lime glass.

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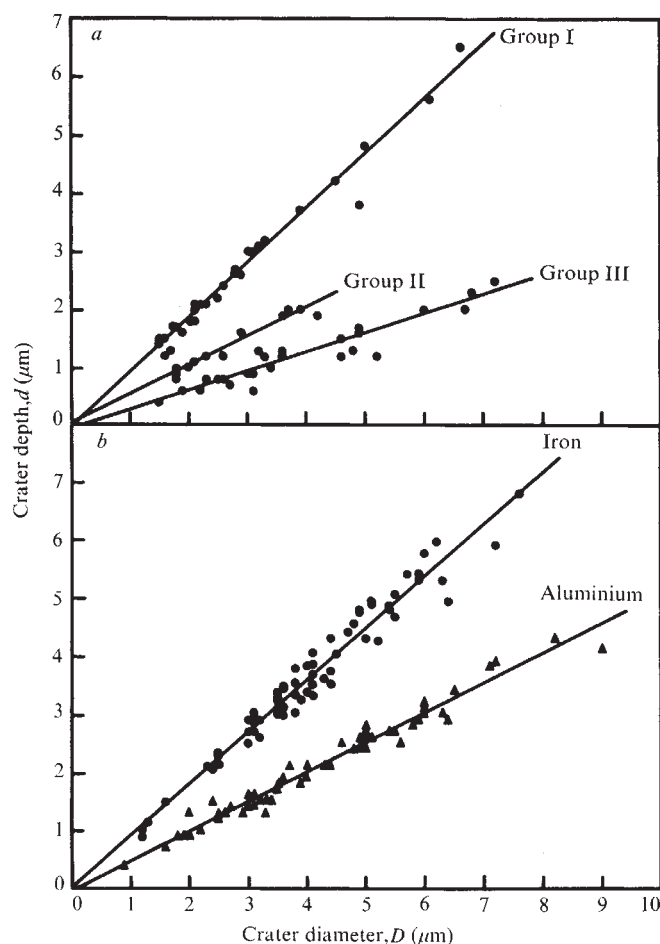


Fig. 2 Depth, d , against diameter, D . The lines are least squares fits to the data points, the slopes of which provide a value for $\bar{\chi}$. *a*, Natural craters on glassy lunar spherules, showing crater Groups I, II and III, with $\bar{\chi} = 0.94 \pm 0.03$, 0.50 ± 0.04 and 0.33 ± 0.02 , respectively; *b*, craters produced by the impact of iron and aluminium projectiles on soda lime glass, with $\bar{\chi} = 0.89 \pm 0.02$ and 0.51 ± 0.01 , respectively.

of differing slope. The slopes of the least squares fits for the three sets of data provide a convenient means of expressing $\bar{\chi}$ for each group, and are in fact those already given. The intercept of each fitted line passes closely through the zero of coordinates; when Groups II and III are considered together the fitted line has a slope smaller than either II or III separately, and a relatively large positive intercept on the d axis. We feel that that is supporting evidence in favour of two separate groups. Figure 2*a* also indicates that the crater diameters in Group II have lower values than either of the other two groups, but this may be because of the relatively smaller number of

samples. The average crater diameters are: Group I, 2.8 μm ; Group II, 2.7 μm ; Group III, 3.8 μm .

On that basis we speculate that the three crater types either result from three groups of micrometeoroids possessing quite different physical properties (for example, iron and stony types), or from three differing velocity groupings of impacting micrometeoroids.

To help us to understand these results we produced craters in soda lime and tektite glasses—chosen because of their similar physical properties to the lunar glasses—by bombardment with iron and aluminium microparticles ($d = 0.5\text{--}6\text{ }\mu\text{m}$ diameter) which were accelerated electrostatically to impact velocities of $1\text{--}7\text{ km s}^{-1}$ using a 2 MV Van de Graaff generator². Velocity selection was not available with this apparatus so the microparticle beam contained a distribution of those velocities, with a most probable velocity of about 3 km s^{-1} . Approximately 60 craters were studied in each case and striking results were obtained when ϕ was plotted against χ . Figure 1*b* shows the histogram peaks for the impact of iron on lunar glass, and aluminium on tektite glass; Fig. 1*c* is for both iron and aluminium projectiles on soda lime glass. The correlation of the iron projectile peaks with the Group I craters is obvious; the aluminium projectile peaks correlate with the low χ groupings, favouring the Group II natural craters. The limited spread in χ for the different glasses suggests that these data can be compared confidently with the natural crater results. Figure 2*b* shows d against D for impacts of both iron and aluminium projectiles on soda lime glass; the slopes of the least squares fits through the points again provide an appropriate value for $\bar{\chi}$. The corresponding data for tektite and lunar glass are of equal quality; the available results for the combinations of projectile and target materials are summarised in Table I.

Iron (density 7.9 g cm^{-3}) was chosen as an appropriate projectile material because it clearly has physical properties similar to iron meteoroids^{3,4} and aluminium was considered suitable because its density (2.7 g cm^{-3}) and melting point are similar to those of the stony (chondritic) meteoroids. Lower density materials in the micron size regime are difficult to obtain but data are available in the literature^{5,6} for the impact of micron-size polystyrene projectiles (density 1.05 g cm^{-3}) in a comparable velocity range ($2\text{--}14\text{ km s}^{-1}$) on soda lime glass. These data show a relatively wide scatter in χ but it has an average value of 0.22, which, taken with the present data, identifies a trend of decreasing $\bar{\chi}$ with decreasing density of the projectile material. Mandeville⁶ has also obtained data for the impact of aluminium projectiles on soda lime glass under conditions similar to those maintained during our work, and he obtained an average value for χ of 0.47, in close agreement with our result.

Nature of impacting particles

Is that sufficient evidence to suggest that the three groups of natural craters originate from physically different micro-

Table 1 Data obtained for craters produced in the laboratory by the impact of microparticles of iron, aluminium and polystyrene on several target materials

Projectile material	Iron (7.9 g cm^{-3})				Aluminium (2.7 g cm^{-3})		Polystyrene* (1.05 g cm^{-3})
	Target material	Lunar spherules	Tektite glass	Soda lime glass	Quartz	Tektite glass	Soda lime glass
$D_m(\mu\text{m})^\dagger$		3.00	3.11	4.47	2.79	3.3	4.22
$\bar{\eta}^\ddagger$		2.04	2.11	3.04	1.89	1.40	1.80
$\bar{\chi}^\S$		0.91		0.89		0.48	0.51
							0.22

* Data for polystyrene from refs. 5 and 6.

$^\dagger D_m$, Mean crater diameter.

$^\ddagger \bar{\eta}$, Mean crater diameter to particle-diameter ratio.

$^\S \bar{\chi}$, Mean crater depth-diameter ratio.

meteoroids? The greatest weakness of this argument is the lack of information on the functional variation of χ with impact velocity and the doubt about whether laboratory impact velocities are appropriate to micrometeoroid lunar impacts. The consensus is that the impact velocities of lunar micrometeoroids lie in the range 5–20 km s⁻¹ (refs 1 and 7–9, and Johnson, J. H., *et al.*, unpublished). Thus, we feel that our impact velocities are acceptable, and the small amount of information concerning the variation of χ with impact velocity tends to show only a weak velocity dependence of χ in the appropriate velocity range⁹. Thus, it may well be that the broadening of the ϕ against χ histogram peaks (Fig. 1) is partly because of the finite velocity spectrum both in the natural and laboratory produced craters.

On the basis of the experimental evidence and reasoning already discussed here, we suggest that the three natural crater groupings result from the impacts of micrometeoroids of distinctly different physical properties. Group I craters with the high $\bar{\chi}$ values—the least contentious case—we ascribe to high density particles; most probably iron micrometeoroids. Groups II and III we ascribe to the impact of low density microparticle material such as the stony and carbonaceous chondrite material, or even ice crystals¹⁰. Further deductions can be made by reference to Fig. 3 in which the densities of the laboratory iron, aluminium and polystyrene microparticles are plotted against the χ values of the laboratory craters produced on soda lime, tektite and lunar glasses. The curve has been fitted through the origin of the coordinates. If the presented data are interpreted literally, then Group II of the natural craters would seem to be formed by micrometeoroids with effective densities in a limited range, centred about 3 g cm⁻³. On this evidence the Group III natural craters are apparently formed by particles with densities of 1–2 g cm⁻³ (allowing for errors of extrapolation) and it is possible that they may result from very low density porous material ('spongy' micrometeoroids). It has often been speculated that very low density material forms a significant component of micrometeoritic material^{11,12} and the present data suggest that about 40% of the total flux of the micrometeoroids in this particular particle size range are of this low density (Group III). We consider that of the 71 natural craters, 33 can be assigned to Group I (iron type), 12 to Group II and 26 to Group III. It follows, therefore, that about 40–50% of the particle flux is iron micrometeoroids.

An indication of the mass distribution between the three groups can be obtained if the micrometeoroid sizes can be

estimated. Laboratory experiments have shown that the average ratio, η , of crater diameter to projectile diameter, is related to the density of the projectile material, but is essentially independent of velocity. That η is independent of velocity is supported by McDonnell *et al.*¹³ who studied the impact of iron projectiles on lunar rock in the velocity range 3–11 km s⁻¹. Table I summarises the averaged values of η ($= \bar{\eta}$) obtained for the impact of the microparticles of the three different materials on several target materials. For the glassy lunar spherules, data for iron projectiles only is available but, as can be seen (Table 1), the value of η is closely similar to that for the impact of iron projectiles on (the very similar) tektite glass. Therefore, we have assumed that glassy lunar spherules and tektite glass behave in the same way under bombardment by particles. Data for polystyrene projectiles are only available for soda lime glass and so we have deduced a value of $\bar{\eta}$ for polystyrene on to glassy spherules. The value of $\bar{\eta}$ for iron on tektite glass, and glassy spherules, is about 75% of that for iron on soda lime glass (Table 1). The same percentage also applies to aluminium projectiles on to these two target materials; itself an interesting result. We therefore assume that this same fraction applies to polystyrene projectiles on to soda lime and tektite glasses, resulting in a value of η for polystyrene onto tektite, and thus lunar glass, of about unity. Thus, we estimate that for lunar glasses the appropriate values for $\bar{\eta}$ are: $\bar{\eta}_{Fe} \approx 2.1$; $\bar{\eta}_{Al} \approx 1.4$; $\bar{\eta}_{polysty} \approx 1.0$. For these deduced ratios we estimate that the mean micrometeoroid diameters, D_m , are: Group I, $D_m = 1.3 \mu\text{m}$; Group II, $D_m = 2.0 \mu\text{m}$; Group III, $D_m = 3.8 \mu\text{m}$. It follows, therefore, that the mass distribution can be estimated.

The result indicates that about 20% of the total mass of the micrometeoroid material is associated with Group I (iron) micrometeoroids, and that the remaining 80% is divided between the low density (stony) Groups: II (10%) and III (70%). That is not an unreasonable result when compared with other estimates of the meteoric material in the Solar System⁴. That the large percentage of the mass of micrometeoritic material apparently exists in the low density form is, however, of considerable interest, although the results indicate that the density does not fall towards the very low densities inferred by Verniani² who observed radio meteors (mean density $\approx 0.3 \text{ g cm}^{-3}$). Radio meteors are, however, substantially larger, and it does not seem unreasonable to speculate that if such relatively large pieces of 'spongy' material disintegrate to produce smaller micrometeoroids, then somewhat higher density particles may result. It should be noted, however, that very shallow microcraters ($\chi \leq 0.1$) would be very difficult to recognise using our techniques, so craters produced by very low density material may be undetectable.

It could be argued that the low χ Group (III) craters are formed by projectiles of lunar origin, that is, ejecta fragments with velocities lower than the lunar escape velocity, $\leq 2 \text{ km s}^{-1}$. Hartung *et al.*¹ have discussed in some detail the possible role of ejecta and they concluded on the basis of estimated relative frequencies of impact and velocity spectra of both ejecta fragments and micrometeoroids, that most microcraters, especially those containing melted host material (the type of craters we examined) are attributable to the impact of micrometeoroids. Also, no spherules with diameters smaller than several tens of microns have been found in these lunar soils, which suggests that ejecta of micron size is not produced in significant amounts. We therefore conclude that Group III craters are caused by micrometeoroids.

In similar studies of depth-diameter ratio Brownlee *et al.*⁹ apparently obtained significantly different results. They studied 68 craters with diameters of 0.2–100 μm and found no obvious dependence of χ on crater diameter (as in all of our studies). They did, however, find a larger dispersion in χ among the smaller craters ($< 4 \mu\text{m}$), but they suggested that this may have been because of increased measurement difficulties for the smaller features. They therefore considered the depth-diameter ratio of only those craters with diameters greater

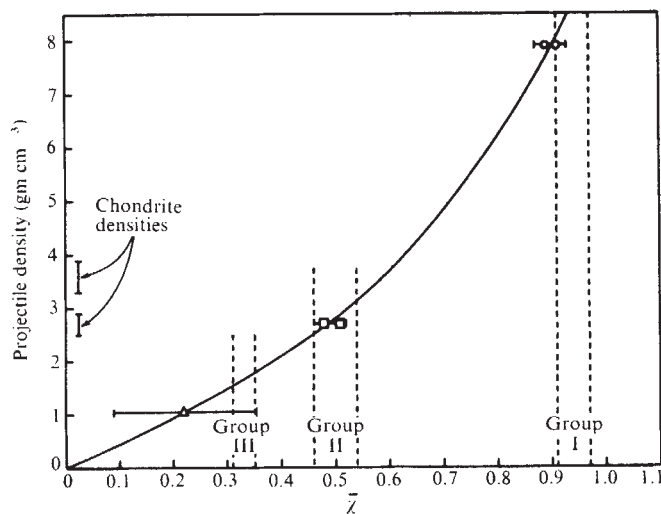


Fig. 3 Plot of projectile density against $\bar{\chi}$ for artificially accelerated microparticles. ○, iron; □, aluminium; △, polystyrene; from the data given in Table 1. The horizontal error bars represent the standard error of the mean. The vertical dashed lines indicate the spread in χ of the Groups I, II and III natural craters on the lunar spherules.

than 4 μm (34 craters), and the resulting histogram of ϕ against χ shows an obvious peak at $\chi = 0.7$. They concluded that this represented evidence against significant numbers of micrometeoroids with densities as high as iron; the majority of particles had densities of 2–4 g cm^{-3} . They also concluded that the absence of very shallow craters ($\chi < 0.2$) suggests that projectiles of very low density ($< 1 \text{ g cm}^{-3}$) are nonexistent or very rare. This very significant result is in complete accord with our data (Fig. 1a) but is in sharp contrast to most meteoroid deceleration data¹². Their major deduction concerning the density of microparticles seems at variance with our result. A plausible explanation of this apparent discrepancy is, however, possible when it is seen that the two sets of data refer to significantly different mean crater diameters (our work: 3 μm ; Brownlee *et al.*: 19 μm) and we are, therefore, dealing

Table 2 Data relating to the natural microcraters observed on the sample of glassy lunar spherules

	Group I	Group II	Group III
Number of craters observed	33	12	26
D_m (μm)	2.8	2.7	3.8
$\bar{\chi}$	0.94	0.50	0.33
Deduced density (g cm^{-3})	8	3	1–2
Deduced η	2.1	1.4	1.0
Deduced range of particle diameters (μm)	0.7–3.1	1.3–2.9	1.5–7.2
Deduced mass range ($\text{g} \times 10^{12}$)	1.5–130	3.3–36	2.7–290

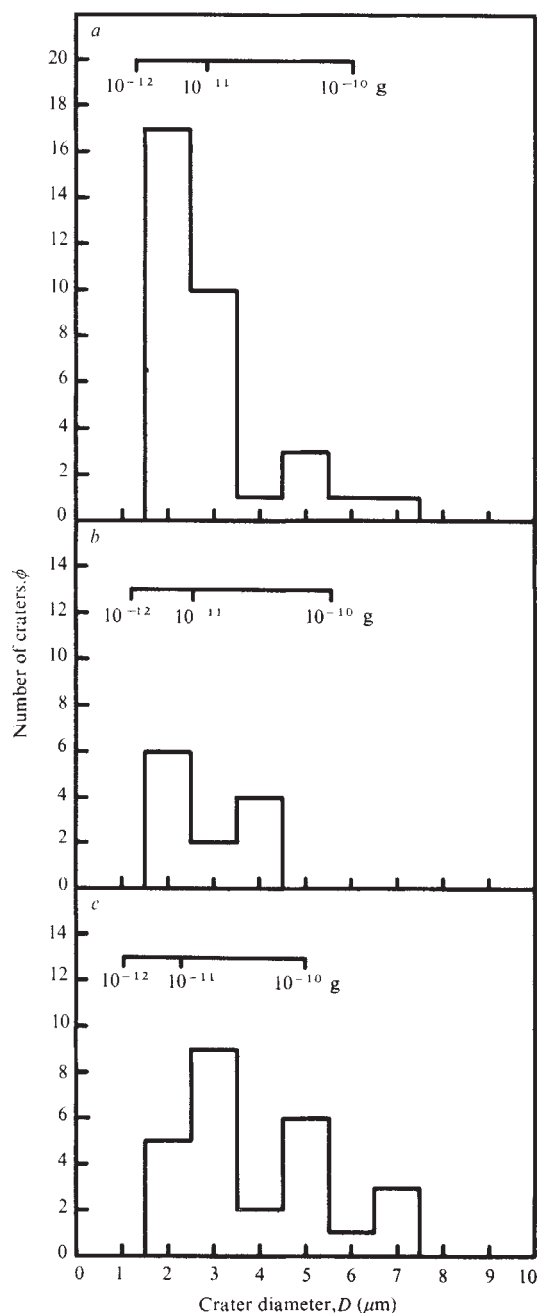


Fig. 4 The numbers of natural craters observed on glassy lunar spherules as a function of the crater diameter for Groups I, II and III (Fig. 1). The equivalent micrometeoroid masses calculated from the inferred material densities are indicated for each group. a, Group I ($\bar{\chi} = 0.94$); b, Group II ($\bar{\chi} = 0.50$); c, Group III ($\bar{\chi} = 0.33$).

with micrometeoroids in quite separate size and mass ranges. Using an $\bar{\eta}$ of 1.4 we estimate that the average micrometeoroid size appropriate to the Brownlee *et al.* data is about 14 μm , and so in the case of the low density particles (2–4 g cm^{-3}) we are dealing with an average difference in mass of about 100 times between the two sets of data.

Figure 4 shows the ϕ against D histograms for the three groups of natural craters. A very rapid increase in ϕ with decreasing D is apparent for the Group I iron micrometeoroids (Fig. 4a). This, coupled with the low density Group III data (Fig. 4c) which, within the statistical fluctuation, indicate a much slower increase in ϕ with decreasing D , strongly suggests an increasing contribution of iron type micrometeoroids to the total micrometeoroid flux in the micron size region. Little can be said about the Group II data (Fig. 4b) except that they do not seem to conflict with the general conclusions drawn for the other two groups. Accordingly, we suggest that the nature of micrometeoritic material is strongly dependent on the region of the mass spectrum being considered, the proportion of low density stony material decreasing and the proportion of high density iron particles increasing with decreasing particle size in the 0.5–8 μm diameter range. This conclusion is most forcibly demonstrated in Fig. 5 in which the absolute differential fluxes of the Groups I and III micrometeoroids are plotted against mean particle diameter. The three points on each curve were obtained by summing the total number of craters observed in the intervals of crater diameter 1.5–3.5 μm , 3.5–5.5 μm , and 5.5–7.5 μm , and deducing the mean particle diameters using the appropriate values of $\bar{\eta}$ (Table 1). The absolute differential flux was obtained from a consideration of the total number of particles observed in each group, the scanned area of the lunar spherules and the estimated exposure time to micrometeoroid bombardment. The last was estimated using the technique¹⁴ of charged particle track analysis. The detailed form of the two curves cannot be interpreted too literally because of the limited amount of data from which they were derived. We believe, however, that the gross features are significant, and indicate that for a particle diameter in excess of about 2 μm , the major fraction of the micrometeoroid flux consists of low density material. The reverse seems to be the case for particle diameters approaching 1 μm , where the high density microparticles predominate. This most interesting feature of the data is quite consistent with the often predicted, but to our knowledge never observed, effects of solar radiation pressure, which acts against solar gravity to exclude very small particles from the Solar system. This pressure is differential in that it acts to a greater extent on low density materials and is thus expected to influence the flux of the Group III micrometeoroids to a greater extent than those of Group I. The magnitude of the radiation pressure effect is traditionally referenced to the particle mass for a given density of material, and so the equivalent mass scales for the two groups—which are not common because of the different material densities of the two groups—are also included in Fig. 5. These results were obtained from simple classical theory¹⁵. Thus, for 2 μm diameter particles the effect is predictably severe on the Group III particles and the reducing slope of the Group III curve of Fig. 5 probably reflects the increasing effect with decreasing size (mass). The

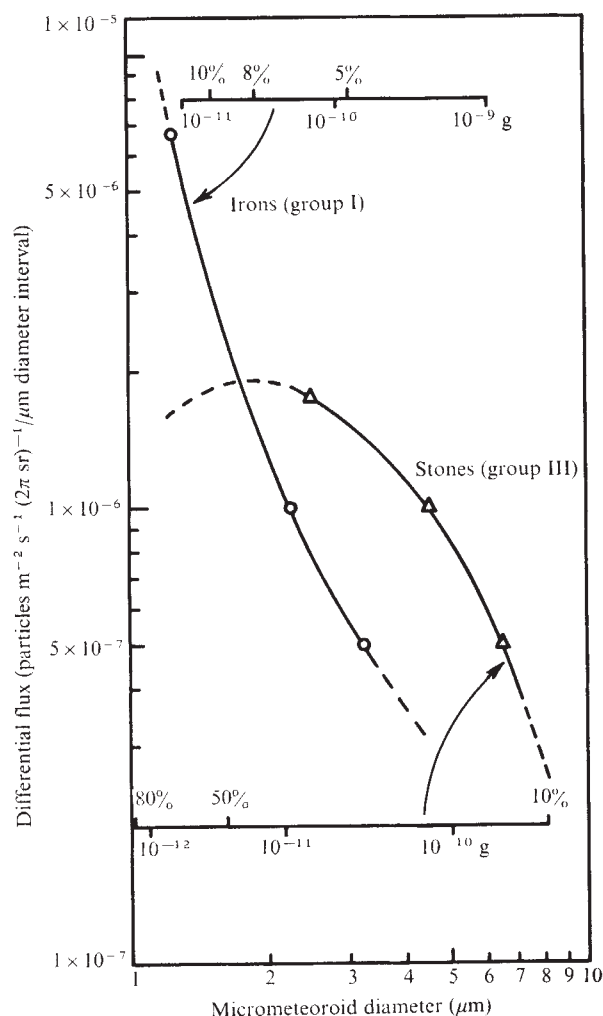


Fig. 5 Differential micrometeoroid flux as a function of micrometeoroid diameter for irons (Group I) and stones (Group III). The equivalent mass scale and a percentage index of the effect of solar radiation pressure are also indicated for each group. The percentages indicate the magnitude of the outward force resulting from solar radiation, relative to that of solar gravity at 1 AU. Thus, at 50% the former is half of the latter.

extrapolation of the curve (dashed) is simply a recognition of the fact that when the radiation pressure effect exceeds 100% (that is, exceeds gravity) then a 'cut off' results and no particles below a given mass ($\approx 4.10^{-13}$ g) or diameter ($0.8 \mu\text{m}$ for a material density of 1.5 g cm^{-3}) can exist in stable orbits in the Solar System. For Group I particles of $2 \mu\text{m}$ diameter, radiation pressure effects are small (a few percent) and thus the differential flux of iron micrometeoroids continues to increase even at very small particle diameters, until the classical 'cut off' at about $0.2 \mu\text{m}$ (2.10^{-14} g) is approached.

The number of craters which we observed per cm^2 of the lunar glasses agrees well with that of Brownlee *et al.*⁹: (both samples were from the Apollo 15 sites). We found approximately 3×10^3 craters cm^{-2} in the diameter range $1.5\text{--}7.2 \mu\text{m}$ whereas the Brownlee *et al.* found 7×10^3 cm^{-2} in the same diameter range. They found an increasing incidence of small craters with decreasing crater diameter, with the cumulative crater surface number density approximately 5×10^4 cm^{-2} at a crater diameter of $0.3 \mu\text{m}$. An extrapolation (on a $\log \phi$ against $\log D$ plot) of our Group I data (Fig. 4a) to a crater diameter of $0.3 \mu\text{m}$ (that is, to particle diameters near to the classical solar radiation pressure limit) indicates a cumulative number density of $\sim 7 \times 10^4$ craters cm^{-2} . The similarity of these estimates tends to indicate that the smaller features in the Brownlee *et al.* data were produced by the impact of iron

micrometeoroids, because similar extrapolation of the Group III data (stony types) indicates much smaller number densities for crater diameters of less than $1 \mu\text{m}$.

Of much greater interest in meteorite studies is the cumulative flux of micrometeoroids at any given mass, and how this parameter varies with mass. The cumulative flux of all micrometeoroids above a mass of 10^{-11} g is indicated by the present data to be approximately $2 \times 10^{-5} \text{ m}^{-2} \text{ s}^{-1} (2\pi \text{ sr})^{-1}$ using the derived exposure time to meteorite impact of $\sim 5.10^4$ yr (ref. 14). This result compares very favourably with the satellite data of Alexander *et al.* reported by Hughes¹⁶, which suggests a value of $\sim 5 \times 10^{-5} \text{ m}^{-2} \text{ s}^{-1} (2\pi \text{ sr})^{-1}$ at 10^{-11} g. The very great difference in the effective sampling time between the satellite experiments (~ 1 yr) and the present 'crater technique' seems to suggest that the micrometeoroid flux has remained sensibly time invariant during the recent history of the Solar System.

A further important result is obtained if the cumulative crater data at the classical solar radiation pressure limit (obtained by applying the extrapolation procedure to the Group I data) is converted to cumulative micrometeoroid flux. The value thus indicated at a micrometeoroid mass of 10^{-14} g is approximately $10^{-3} \text{ m}^{-2} \text{ s}^{-1} (2\pi \text{ sr})^{-1}$. If the mass limit of solar radiation pressure for iron micrometeoroids were lower than that predicted by the classical theory, then it is likely that relatively large fluxes of very small iron micrometeoroids ($d < 0.1 \mu\text{m}$) could exist in stable orbits in the Solar System. Highly sensitive detectors would be necessary to verify the existence of such small particles, such as the plasma micrometeoroid detector¹⁷ incorporated into the Prospero satellite¹⁸ which did, in fact, indicate a total cumulative flux at its predicted threshold mass sensitivity of 10^{-15} g in the range 10^{-2} to $10^{-1} \text{ m}^{-2} \text{ s}^{-1} (2\pi \text{ sr})^{-1}$.

Our study indicates that the differential flux of iron type micrometeoroids increases rapidly as their size reduces progressively to diameters below about $2 \mu\text{m}$. For micrometeoroid diameters $\gtrsim 2 \mu\text{m}$, micrometeoroids with a density of $1\text{--}4 \text{ g cm}^{-3}$ increasingly contribute to the flux, until they represent in excess of 90% of the total. The available evidence indicates that the major component of the flux at this low density grouping is of material in the density range $1\text{--}2 \text{ g cm}^{-3}$ with a smaller component at a density near to 3 g cm^{-3} . Thus, the data seem to support the accepted view that iron type meteoroids represent only a very small fraction of the total mass of meteoritic material. In the diameter range $0.1 \mu\text{m}\text{--}1.0 \mu\text{m}$, however, a large fraction of the total flux of micrometeoroids must be of the high density iron type and their cumulative flux at the lowest mass may approach the relatively high values indicated by the most sensitive micrometeoroid detectors.

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Partial nucleotide sequence of a prokaryote initiator tRNA that functions in its non-formylated form

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When grown in the absence of folate, Streptococcus faecalis initiates protein synthesis with non-formylated methionyl-tRNA^{Met}. The nucleotide sequence of this tRNA differs from that of tRNA^{Met} synthesised in the presence of folate — which functions as N-formyl-methionyl-tRNA^{Met}—only in the 'GTΨC' loop, where uracil is found in the tRNA^{Met} of folate-free cells in place of thymine.

Initiation of protein synthesis in the bacterium *Streptococcus faecalis* R is an interesting exception to the generalisation concerning the difference in this process in prokaryotes and eukaryotes. Initiation of protein synthesis in all prokaryotes and certain organelles of eukaryotes appears to involve a special tRNA, tRNA^{Met}, that functions as a formylated methionylated derivative, N-formyl-methionyl-tRNA^{Met}, and donates a formyl-methionyl residue to the N-terminus of the peptide chain^{1–3}. The formyl group essential for the initiation process is transferred to methionyl-tRNA^{Met} from a derivative of folic acid, 10-formyltetrahydrofolate, by means of a specific enzyme^{4,5}. Initiation of protein synthesis in the cytoplasm of eukaryotes also involves a unique tRNA^{Met} species. However, formylation of eukaryotic methionyl-tRNA^{Met} does not occur *in vivo*, although methionyl-tRNA^{Met} from some eukaryotes can be formylated *in vitro* by the transformylase of *Escherichia coli*².

S. faecalis cannot synthesise folate or its derivatives *de novo* and normally requires the presence of one of these substances in the growth medium. The organism, however, can grow at essentially the normal rate in the absence of any of these compounds when the growth medium contains serine, methionine, thymine, a purine base and pantothenate⁶, each substance a product of a synthetic pathway involving a folate derivative^{7,8}. N-formyl-methionyl-tRNA^{Met}, considered to be essential for initiation of protein synthesis in bacteria^{1–3}, is also a product of an enzymatic reaction involving a folate derivative^{4,5}. However, it is not necessary to supply the organism with this substance when it is grown in folate-free medium. Samuel *et al.*^{9–11} demonstrated that under these growth conditions, initiation of protein synthesis proceeds with non-formylated methionyl-tRNA^{Met} and appears in this respect to resemble the protein initiation process in eukaryotes. Their data showed that the ability of this bacterium to initiate protein synthesis with non-formylated methionyl-tRNA^{Met} is attributable to an alteration in the tRNA^{Met}, and not to a modification of the ribosomes or factors involved in protein initiation. The tRNA^{Met} of *S. faecalis* grown in the absence

of folate can be distinguished from the tRNA^{Met} of *S. faecalis* grown in the presence of folate by several physical, chemical and biological criteria. Of particular significance is the observation that non-formylated methionyl-tRNA^{Met} from minus-folate cells forms an initiation complex with ribosomes, poly (A,U,G), and initiation factors under conditions where methionyl-tRNA^{Met} from plus-folate *S. faecalis*, like other prokaryotic initiator tRNAs, requires prior formylation for formation of the initiation complex¹¹.

Sequence analyses have been undertaken in several laboratories in efforts to identify structural features of the tRNA^{Met} species that are responsible for their different biological properties. The nucleotide sequences of two iso-species of tRNA^{Met} and two of tRNA^{Met} from *E. coli* have been determined^{12–14}. More recently the sequences of the tRNA^{Met} from yeast¹⁵ and three mammalian sources^{16,17} have been reported. The tRNA^{Met} sequences of the mam-

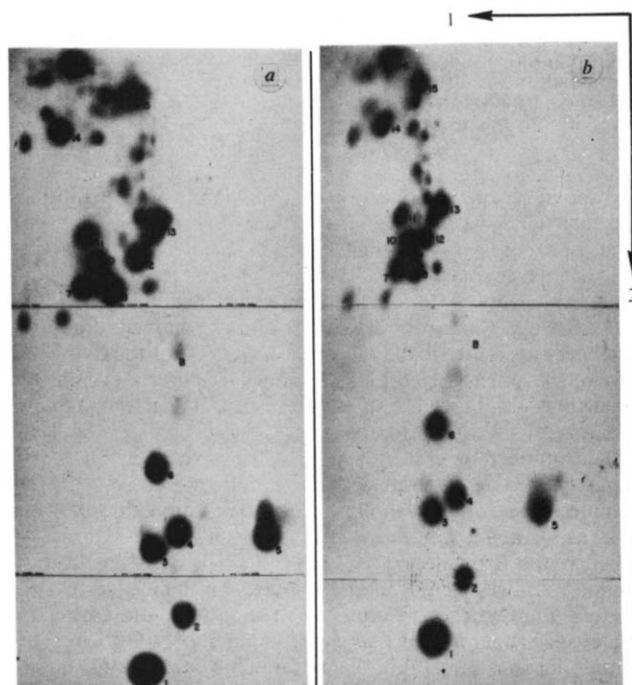
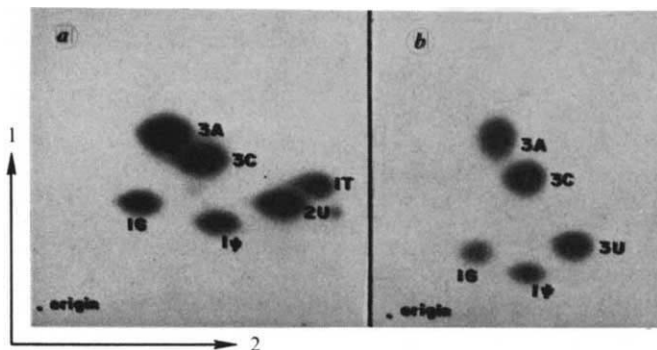


Fig. 1 Autoradiograph of the two-dimensional fractionation of the U₁ RNase digestion products of (a) plus-folate and (b) minus-folate *S. faecalis* tRNA^{Met}. (1) First dimension: cellulose acetate, pH 3.5; (2) second: DEAE-cellulose, 7% formic acid. B indicates position of standard blue marker¹⁸.



malian cells are identical and show a high degree of homology with *E. coli* tRNA_f^{Met}, as well as with yeast tRNA_f^{Met}, that is suggested to reflect the special constraints in evolutionary divergence that the unique role of this tRNA has imposed. An unusual feature of eukaryote tRNA_f^{Met} is the lack of a GTΨC sequence in loop IV¹⁵⁻¹⁷. It was thus of particular interest to note that the tRNA_f^{Met} of *S. faecalis* grown in the absence of folate does not contain ribothymidine (T), whereas tRNA_f^{Met} from cells grown with folate does contain this modified nucleoside¹¹. We have therefore investigated the more detailed chemical structure of tRNA_f^{Met} of *S. faecalis*.

S. faecalis was grown in the presence (plus-folate) or in the absence (minus-folate) of folic acid in a low phosphate medium containing ^{32}P -phosphate, a modification of a medium described previously¹⁰. Cells were collected by centrifugation and then broken by sonication. Unlabelled carrier tRNA was added and the tRNA_f^{Met} was isolated and purified essentially as described elsewhere¹¹. The final tRNA_f^{Met} sample was estimated to be 80–90% pure.

The tRNA^{Met} was analysed as described by Barrell¹⁸, except that U₁ RNase, a gift of Drs A. Blank and C. A. Dekker, was used instead of T₁ RNase and frequent use was made of the two-dimensional thin-layer chromatography system for nucleotides described by Nishimura¹⁹. The detailed data of this investigation will appear elsewhere. As suggested by Barrell¹⁸, except where stated otherwise, the letters A, C, G, and U are used for the 3' nucleotide residues and a sequence such as ACG represents ApCpGp. Parentheses enclosing letters indicate that the sequence of these nucleotides is unknown.

Two-dimensional thin-layer chromatography¹⁹ of this 3' nucleotides obtained on enzymatic digestion showed that, in addition to the four major nucleosides, plus-folate tRNA^{Met} contains one T, one pseudouridine (ψ), and one dihydrouridine (hU), whereas minus-folate tRNA^{Met} contains only one ψ and one hU.

As Fig. 1 shows, the fingerprints of the oligonucleotides obtained by digestion of plus-folate and minus-folate tRNA^{Met} with U₁ RNase were essentially identical, indicating that the sequence of the two tRNAs are similar. Analysis of the oligonucleotides showed that the compositions of corresponding fragments were the same for both tRNAs except for oligonucleotides 16 (designated U-16) which, as seen in Fig. 2, was shown by two-dimensional thin-layer chromatography to have a composition (TψU₂C₄A₃)G in plus-folate tRNA^{Met} and (ψU₃C₃A₃)G in minus-folate tRNA^{Met}, suggesting that in the latter case a single U residue had not undergone methylation to produce T (5-methyluridine).

As Fig. 3 shows, fingerprints obtained on pancreatic

RNase A digestion were also quite similar for the two tRNAs, establishing the sequence similarity. In plus-folate tRNA^{Met}_f, T was found in oligonucleotide 12 (P-12), the composition of which was shown to be (AG₂)T. The corresponding oligonucleotide from minus-folate tRNA^{Met}_f had the expected composition (AG₂)U. All other oligonucleotides examined had identical composition for the two tRNAs. Attention was thus focused on the sequence surrounding T from plus-folate tRNA^{Met}_f and the corresponding sequence from the minus-folate tRNA^{Met}_f.

On further digestion of the U-16 oligonucleotides with pancreatic RNase, both the plus-folate and minus-folate fragments yielded one equivalent of A₃U, one equivalent of G, and three equivalents of C; however, the plus-folate sample yielded an equivalent each of T, ψ , and U, whereas the minus-folate sample gave one equivalent of ψ and two equivalents of U. On partial digestion with U₂ RNase, both samples yielded (U₂C₃)G, A₃, A₂ and A fragments. However, plus-folate U-16 yielded the oligonucleotides (T ψ CA) and (T ψ C), whereas the corresponding minus-folate fragments were (U ψ CA) and (U ψ C). After removal of the 3' phosphate from the unexpected but consistently obtained U₂ RNase digestion product (U ψ C), pancreatic RNase digestion produced U and ψ , indicating that C_{OH} was at the 3' end of the dephosphorylated fragment, this base having been released as the nucleoside and thus undetected by autoradiography. From these data one can deduce the sequence (T ψ)CAAAU(CCU)G for the oligonucleotide U-16 from plus-folate tRNA_I^{Met} and (U ψ)CAAAU(CCU)G for the corresponding oligonucleotide from minus-folate tRNA_I^{Met}.

The final sequence was derived by analysis of the pancreatic RNase product P-12 from plus-folate tRNA_f^{Met}, the composition of which is (AG₂)T. The T is automatically assignable to the 3' end by nature of pancreatic RNase specificity. After removal of the 3' phosphate, snake venom phosphodiesterase treatment yielded pG and pT in the ratio 2: 1, thus establishing the sequence as AGGT. Since there is only one T in plus-folate tRNA_f^{Met}, oligonucleotides P-12 and U-16 must overlap and T must be at the 5' end of the oligonucleotide U-16. Figure 4 shows the derived sequence of what is apparently the 'GTψC' loop (IV) and stem of tRNA_f^{Met} of *S. faecalis*.

This sequence bears striking resemblance to the 'GT ψ C'

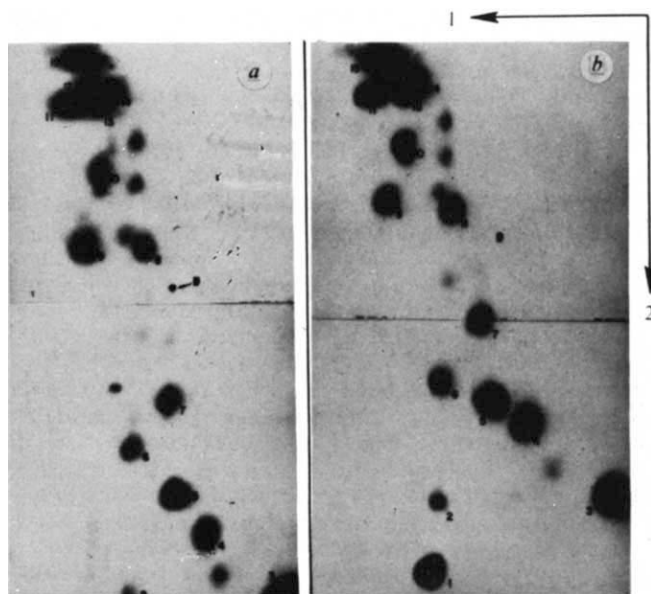


Fig. 3 Autoradiograph of two-dimensional fractionation of pancreatic RNase digestion products of (a) plus-folate and (b) minus-folate *S. faecalis* tRNA^{Met}. (1) and (2) as in Fig. 1. B indicates position of blue marker.

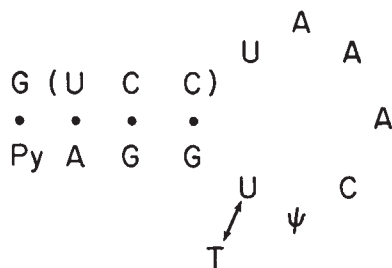


Fig. 4 Nucleotide sequence of the presumed loop IV and stem of tRNA^{Met} from minus-folate *S. faecalis* (primary sequence) and tRNA^{Met} from plus-folate cells (T replacing U).

arm of tRNA^{Met} of *E. coli*¹³, the only difference being that in the *E. coli* tRNA a G-C pair is found in place of the presumed U-A base pair in the stem of the *S. faecalis* tRNA. Recently it has been shown that the single-stranded portion of the 'GTψC' arm of *Anacystis nidulans* tRNA^{Met} has this same base sequence²⁰.

The similarity in the U₁ RNase digestion products of the plus-folate and minus-folate tRNAs in combination with the similarity of their pancreatic RNase digestion products indicates that the remainder of the nucleotide sequence is identical for the two *S. faecalis* tRNAs. Although the complete sequence of tRNA^{Met} of *S. faecalis* has not been determined, a comparison of its enzymatic digestion products with those of tRNA^{Met} of *E. coli*¹² indicates that there is a great deal of similarity between these two molecules.

Like tRNA^{Met} of *E. coli*, the *S. faecalis* tRNA has pCG (oligonucleotide U-7) at the 5' terminus. It is not known, however, if the terminal C is hydrogen bonded to a G residue since there is uncertainty about the sequence of the 3' end of the tRNA. Oligonucleotide U-5, which has the composition (A₂C₃), is identifiable as the 3' terminus by its lack of G. Presumably it contains an undetectable A_{OH} and has the sequence (CA₂)CCA_{OH}. The composition of this oligonucleotide is identical to that of the fragment CAACCA_{OH} obtained upon T₁ RNase digestion of tRNA^{Met} from *E. coli*¹². Oligonucleotide U-5 migrates differently, however, in the second dimension from what was reported for CAACCA_{OH}¹². Although the mobility of U-5 is similar to that reported for CCACCA_{OH}²⁰, reproducible quantitative data indicate that this is not the composition of the *S. faecalis* fragment. The 5' terminal residue of *S. faecalis* tRNA^{Met}, like that of tRNA^{Met} of *E. coli*¹³ and *A. nidulans*²⁰ and unlike that of most other tRNAs examined, is not hydrogen bonded to a residue four nucleotides removed from the 3' A_{OH}. It is not known at this time, however, if the 3' terminus of *S. faecalis* tRNA^{Met} is identical to or similar to that of *E. coli* tRNA^{Met} or if the 5' C is hydrogen bonded to a G that is more than four nucleotides removed from the 3' A_{OH}.

At least a portion of the hU loop appears identical for *E. coli* and *S. faecalis* tRNA^{Met}; namely, both have a single hU which occurs in the sequence GGhUAG¹³. (The sequence of the *S. faecalis* oligonucleotide P-11 is GGhU, that of U-9 is hUAG.) *S. faecalis* tRNA^{Met} on U₁ RNase digestion yields (C₂U)G (U-12) and on pancreatic RNase digestion AGC (P-7). These two could be from the hU loop and would be consistent with a sequence identical to that of the *E. coli* tRNA, namely CAGCCUGGhUAG¹³, the first and last bases forming a hydrogen bond.

Oligonucleotide U-15 has the composition (A₃C₅U₂)G and may be from the anticodon loop since *E. coli* tRNA^{Met} on T₁ RNase digestion yields the anticodon-containing oligonucleotide 2'OMeCUCAUAACCCG¹². The pancreatic RNase product P-13 from *S. faecalis* has the sequence GGGC. These two products would be consistent with an anticodon loop and stem similar to that of the *E. coli* tRNA with the notable exception that tRNA^{Met} of *S. faecalis* does

not have 2'OMeC. Furthermore, although both isospecies of tRNA^{Met} from *E. coli* contain 4-thiouracil and one contains 7-methylguanine¹³, tRNA^{Met} from plus-folate *S. faecalis* has no modified bases other than T, ψ, and hU and tRNA^{Met} from cells grown in the absence of folate has only ψ and hU. *S. faecalis* tRNA^{Met} has the smallest number of modified bases observed among tRNAs.

Analysis of unfractionated and partially fractionated *S. faecalis* tRNA showed that the absence of T is not unique to the tRNA^{Met} species. Although T is present in tRNA from plus-folate cells, this modified residue is absent in all tRNA species of *S. faecalis* when it is grown in folate-free medium.

In summary, tRNA^{Met} of *S. faecalis* is similar in sequence to tRNA^{Met} of *E. coli*, although *S. faecalis* tRNA^{Met} lacks several modified bases found in the *E. coli* species. When grown in the presence of folate, *S. faecalis* tRNA^{Met} contains the modified base T in the sequence GTψC. When the bacterium is grown in the absence of folate, methylation of the specific U residue does not occur, the resulting sequence being GUψC. The remainder of the nucleotide sequence is identical for plus-folate and minus-folate *S. faecalis* tRNA^{Met}.

The absence of this single methyl moiety is sufficient to alter the chromatographic and melting properties of the tRNA^{Met} species and, most significantly, to allow the bacterium to initiate protein synthesis with non-formylated methionyl-tRNA^{Met} when 10-formyltetrahydrofolate, an essential component of the formylation reaction, is unavailable. Further investigation is required to determine whether the ability of this tRNA^{Met} to function in its non-formylated form is the consequence of direct interaction of the altered portion of the tRNA with ribosomes or various factors involved in protein synthesis, or whether it is the result of a conformation change in the tRNA molecule. It would be interesting to examine the tRNA^{Met} and protein initiation process in the mutants of *E. coli* which are totally lacking T²¹ and strains of *Bacillus subtilis* which are low in T content^{22,23} to determine if the absence of T in other prokaryotic initiator tRNAs allows them to function in their non-formylated form.

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letters to nature

A Newtonian view of relativistic cosmology

IN spite of the success of Milne and McCrea¹ in using classical mechanics to reproduce the Friedmann universes, the mathematically simplified apparatus of Newtonian cosmology has been of only limited use in exploring relativistic world models. In general, the required formulation of Newtonian universes^{2,3} leads to indeterminate equations of motion: one has an infinite region of homogeneous dust (density $\rho(t)$, pressure $p = 0$), in which the gravitational field cannot be found classically. This letter outlines a way of resolving the indeterminacy, and gaining an insight into relativistic cosmology, through equations that reveal the dynamics of relativistic dust world models in a quasi-Newtonian fashion.

The method is a local-approximation technique using spatial power series expansions in comoving coordinates (ξ^i , τ). We take a cosmological metric

$$ds^2 = d\tau^2 + 2g_{4i} d\xi^i d\tau + g_{ij} d\xi^i d\xi^j \quad (1)$$

and noting the conservation equations $\partial g_{4i}/\partial \tau = 0$, we write

$$g_{4i} = E_i + F_{ir}\xi^r + G_{irs}\xi^r\xi^s + \dots \quad (2a)$$

$$g_{ij} = M_{ij} + N_{ij}^r\xi^r + \frac{1}{2}P_{ij}^{rs}\xi^r\xi^s + \dots \quad (2b)$$

Because the coefficients on the right of (2a) are constant, there is a transformation of τ that gives $E_i = 0$ and $F_{ij} = -F_{ji}$. M , N and P are τ -dependent arrays, but we can set $-M_0 = I$ (the 3×3 identity matrix) and $N_0 = 0$ at an initial instant τ_0 .

A local correlation is then obtained with the usual kinematics of Newtonian universes, in which the velocity v of matter, being a linear function of Cartesian coordinates x^i , is defined by a matrix H depending only on the absolute Newtonian time t . H decomposes into an expansion scalar $\bar{H}$, a traceless symmetric shear tensor Q , and an antisymmetric spin tensor W :

$$v = Hx; \quad H = H(t) = \bar{H}I + Q + W \quad (3)$$

The classical motion is given in Lagrangian coordinates by a matrix $A(t)$. A particle initially at x_0 has the general position $x = Ax_0$, provided that

$$\dot{A} = HA; \quad A_0 = I \quad (4)$$

We can identify the x_0^i locally with the comoving coordinates ξ^i , and in the local approximation we can also interchange t and τ . With these correspondences, the leading coefficients in equations (2a, b) are found to be the matrices

$$F = -A'WA; \quad M = -A'A \quad (5)$$

This local Newtonian interpretation of a general comoving metric is useful in itself.

Although the classical potential U is indeterminate, the Euler equation shows that it may be written as a quadratic form given by a t -dependent symmetric matrix Φ : $U = \frac{1}{2}\Phi_{ij}x^ix^j$; $\Phi = \Phi(t) = \Phi^t$. The Euler, Poisson and continuity equations reduce to

$$\bar{H} + H^2 + \Phi = 0 \quad (6a)$$

$$\text{tr}(\Phi) = 4\pi G\rho \quad (6b)$$

$$\dot{\rho} + \rho \text{tr}(H) = 0 \quad (6c)$$

and here the inadequacy of Newtonian mechanics is obvious: we have just one equation for the six independent Φ_{ij} . In the absence of spherical symmetry extra conditions may be imposed arbitrarily³⁻⁵, but the Φ corresponding to general relativity is far from evident.

But we can get the relativistic answer by extending the local-approximation formalism to Einstein's field equations. With the comoving coordinates chosen locally as before, it turns out that the relativistic potential matrix Φ_r is given by

$$\Phi_r = \frac{1}{3}\pi G\rho I + \bar{H}Q - (Q^2 - \frac{1}{3}\text{tr}(Q^2)I) + \Phi_c \quad (7a)$$

$$\Phi_c = \psi - \frac{1}{3}\text{tr}(\psi)I; \quad \psi = -(A^{-1})'(\bar{R}^0)A^{-1} \quad (7b)$$

where the matrix $\bar{R}^0$ is the local value of the Ricci subtensor $\bar{R}_{ij}$ of the ξ^i 3-spaces. This result shows how matter, motion and spatial curvature contribute to the potential.

The determinate quasi-Newtonian potential with $\Phi_c = 0$ gives an exact description only of universes whose comoving space sections have isotropic curvature: the Bianchi Type I models, a non-rotating subclass of Type V, and the closed Friedmann universe (see ref. 6). But it is interesting that rotating solutions with $\Phi_c = 0$ need not have a singularity. (This is most easily shown for cylindrical models^{5,7}.) We cannot infer here that similar exact relativistic solutions exist. Given the wide scope of the Penrose-Hawking theorems⁸, the implication is rather that curvature anisotropies are a vital factor in causing singularities.

In general the potential remains indeterminate, because Φ_c cannot be predicted. (Though Φ_c can be calculated for homogeneous universes, since their metrics are known in advance.) Nevertheless, the quasi-classical equations are of some heuristic value, and they can be used to classify and elucidate various kinds of singularities and indefinite expansion occurring in relativistic world models. They show in particular why expanding universes that approach isotropy have to be regarded as unusual⁹: the shear tensor Q is self-damping during expansion only in the special cases when Φ_c is insignificant.

The decisive effects that Φ_c may have are exemplified by Bianchi Type VI universes, for which solutions can be generated by computer. Some rotating models of this class exhibit not only anisotropic expansion, but also matter singularities of a 'ribbon' form that seems to be hitherto unreported: the principal axes of a local comoving ellipsoid tend to zero, a constant,

and infinity. The limiting behaviour is apparently of power law type, with the volume proportional to τ^p , where $0 < p < 1$, as $\tau \downarrow 0$. (In isotropic singularities $p = 2$, and velocity-dominated ones¹⁰ have $p = 1$ if a simple power law applies.)

These Bianchi VI singularities are distinct from the 'whimper' sort¹¹, in which the homogeneous 3-spaces of a synchronous coordinate system change their signature. The models referred to here have been studied using a comoving metric of the form (this actually ranges over Types I, III, V, VI)

$$ds^2 = d\tau^2 + 2\lambda\eta d\xi^2 d\tau - \{\alpha(d\xi^1)^2 + 2v\eta d\xi^1 d\xi^2 + \beta(\eta d\xi^2)^2 + \gamma(\zeta d\xi^3)^2\} \quad (8)$$

where $\eta = \exp(m\xi^1)$, $\zeta = \exp(n\xi^1)$; α, β, γ, v are functions of τ ; λ, m, n are constants. (see equations (2). The Newtonian interpretation (5) will usually require a local transformation of the metric (8).) The field equations for (8) show that only exceptional cases, if any, can pass through a whimper singularity (corresponding here to $\alpha\beta = v^2$), and probably this is true in general.

As for matter singularities, the quasi-Newtonian equations suggest a vast range of possible behaviour in relativistic cosmology, making present observations of radiation isotropy appear all the more remarkable. Details of singularity behaviour and other specialised aspects will be discussed elsewhere.

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Redshifts of Markaryan galaxies

MARKARYAN¹ and Markaryan and Lipovetsky² have given data on a few hundred galaxies with strong ultraviolet continua. Markaryan classifies them on the basis of the concentration of the source of the continuum (*s* means concentrated and *d* diffuse objects; *sd* and *ds* are intermediate). Especially among the *s*-type objects there seems to be contamination with QSOs when their spectra, colour and presumable morphology (spheroidal, compact) are considered. Redshifts are now available for many Markaryan galaxies providing new material for studies of the redshift problem. It is worthwhile to see whether the claimed peculiarities in redshifts of QSOs and related emission-line objects^{3–6} have counterparts in the distribution of these

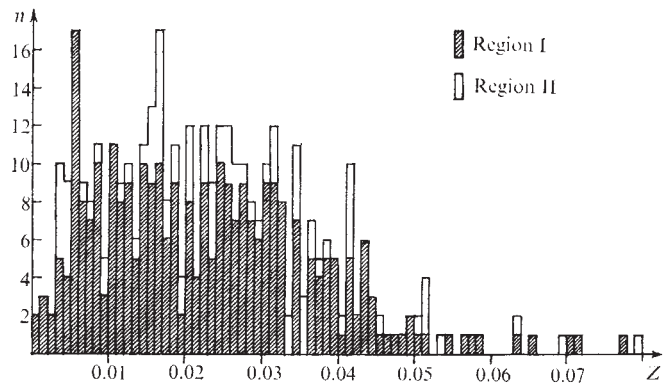


Fig. 1 Distribution of the redshifts of Markaryan galaxies.

smaller redshifts. According to Arp and Sulentic (see ref. 7) many Markaryan galaxies seem to be companions of small-redshift bright galaxies and thus they possibly have anomalous redshifts.

I present here a preliminary survey of the data which consist of 394 redshifts^{8–15} ($z < 0.08$) of which 90, 92, 65, and 147 are for *s*, *sd*, *ds*, and *d* objects, respectively. The measurements of Ulrich¹³ and Sargent¹⁴ were preferred.

The distribution of all the redshifts is presented in Fig. 1, using the interval $\Delta z = 0.001$, corresponding to the accuracy of most redshifts. Burbidge³ and Burbidge and O'Dell^{5,6} found for different kinds of emission-line objects peaks near $z=0.03$ and $z=0.06$. In my analysis no groupings at these values can be seen, probably because of the different class of objects studied.

But there is a peak at $z=0.015$ – 0.016 , with some interesting features. Its statistical significance can be approximated from Poisson's formula when the gross frequency distribution of the redshifts is taken as rectangular between $z=0.003$ and $z=0.030$. The probability that 30 redshifts should fall inside an interval of $\Delta z = 0.002$ is about 0.05. But what really makes such a peak interesting is how its properties are consistent with ideas concerning its origin. One cannot rely on purely statistical arguments because among these small redshifts random motions and cosmological recession tends to smear possible non Dopplerian peaks which thus must be only weakly discernible.

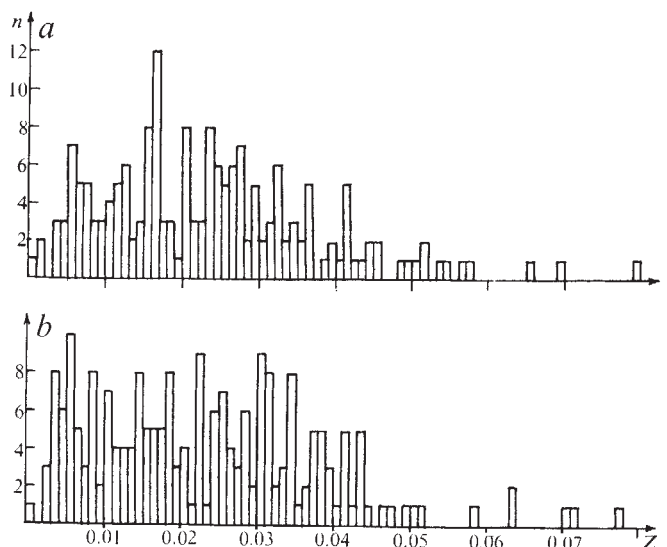


Fig. 2 Distribution of the redshifts of: *a*, *s* and *sd* objects combined; *b*, *ds* and *d* objects combined.

The value $z=0.015-0.016$ is half the value 0.03 and a quarter of 0.06. This might suggest a downward extension of Burbidge's relation³ $z=n \times 0.061$, $n=1, 2, 3, \dots$

Figure 2a shows the redshift distribution for *s* and *sd* objects, and Fig. 2b for the classes *ds* and *d*. Evidently the peak originates mainly from the former ones, which is consistent with their more quasar-related nature.

If the peak is caused by intrinsic redshifts, the objects giving rise to it should be rather near, so that the cosmological redshift would not smooth it off. So it should originate chiefly from objects in the local supergalaxy. The dividing line on the celestial sphere given by Rubin *et al.*¹⁶ separates the low redshift Scl-galaxies (Region I) from the high redshift ones (Region II). Region II includes the central parts of the local supergalaxy. The shaded area in Fig. 1 represents the objects in and near Region I. Region II is chiefly responsible for the peak. Redshifts between $z=0.008$ and $z=0.014$ occur predominantly in Region I (ref. 7). From Fig. 1 it can be seen that at the peak and to the right of it the proportion of Region II redshifts increases.

Also in Arp's list¹⁷ of interacting galaxy groups (five groups), six out of 16 hypothetical intrinsic redshifts (obtained by

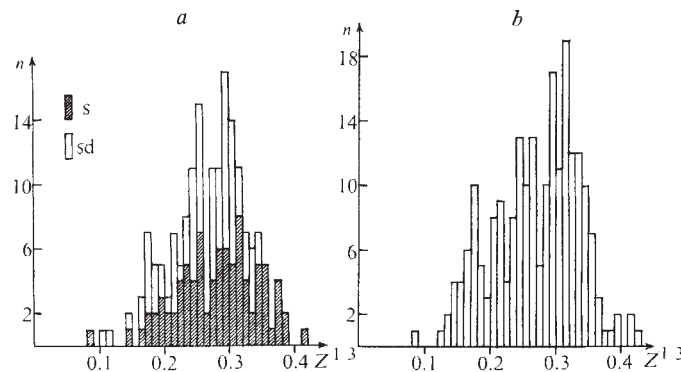


Fig. 3 Transformed ($z \rightarrow z^{1/3}$) distribution of the redshifts of: a, *s* and *sd* objects; b, *ds* and *d* objects combined.

subtracting the redshifts of the primaries) would fall inside the peak $z=0.015-0.016$. Pecker *et al.*¹⁸ have interpreted the redshift distribution of QSOs using the relation

$$z = aRT^3,$$

where a is a constant, R the characteristic dimension of the QSO and T the temperature. The transformation $z \rightarrow z^{1/3}$ leads in the case of QSOs to a symmetric distribution around a plausible value of the temperature. It can be seen from Fig. 3 that the transformation $z \rightarrow z^{1/3}$ leads to a nearly symmetric distribution in the case of *s* and *sd* objects, but for *ds* and *d* objects the resulting distribution is less symmetrical. The small excess of numbers in the left hand side of the distribution of *s* and *sd* objects (Fig. 3a) comes from the peak at $z=0.005$. This peak is seen also for the *ds* and *d* objects.

These data seem to support the view that compact and nearly compact Markaryan objects have anomalous redshifts. This is consistent with the general idea that anomalous redshifts are connected with compact objects.

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Missing mass around galaxies: morphological evidence

RECENTLY we have obtained convincing empirical indications on the considerable role of hidden matter in the dynamics of single and double galaxies¹. It seems that this matter is concentrated around massive galaxies, forming their coronas. The total mass of galaxies is about one order of magnitude greater than the mass of their visible parts.

Massive galaxies like our Galaxy or the Andromeda galaxy, M31, are surrounded by clusters of small companion galaxies². Searches for new effects of galactic coronas have led us to the discovery of a correlation between the morphological types of companion galaxies and their distances from the parent galaxies. It seems that elliptical companions are strongly concentrated around the parent galaxies whereas non-elliptical (spiral and irregular) ones populate preferentially the peripheral regions.

To derive this dependence we have collected the data on the companions of our Galaxy and of three external spiral galaxies—M31, M81 and M101 (refs 2-7). The adopted distances of these galaxies are as follows: 0.69 Mpc, 3.25 Mpc, and 7.25 Mpc (G. Tammann, private communication). The joint distribution (luminosity as function of distance from the parent galaxy) of the companions of all the four galaxies considered is given in Fig. 1. The regions populated by elliptical and non-elliptical companions are very sharply separated and the line of segregation is well defined.

To understand the nature of the regularity discovered we draw attention to the fact that the segregation of companion galaxies is connected essentially with one property—the presence or absence of the interstellar gas in the companion. Spiral and irregular companions, both of them containing much interstellar gas, are located to the right of the segregation line, elliptical companions, containing practically no gas, are situated to the left of this line. A simple estimate shows that the segregation mechanism does not reduce to the tidal interaction which is too weak to produce such radical effects. If we reject the possibility of specific very artificial conditions during the formation of galaxies, there evidently remains the suspicion that the only agent capable of producing the interaction of the galaxies studied is intergalactic matter surrounding massive galaxies.

Let us suppose that the corona of a massive galaxy contains much gas. Consider a companion galaxy having its own gas

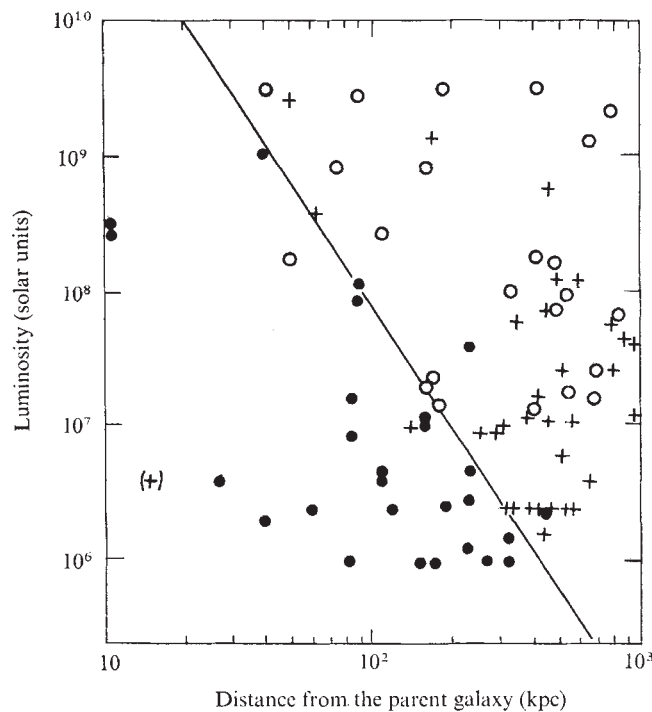


Fig. 1 The distribution (blue luminosity as function of distance from the parent galaxy) of the companions of our Galaxy and of three external galaxies: M31, M81 and M101. Elliptical companions populate the area to the left of the segregation line, non-elliptical companions to the right of this line. The presence of three spiral and irregular companions left of the segregation line is due to the projection effect; two elliptical companions right of the line have apparently more elongated orbits than other companion galaxies. ●, Ellipticals; ○, spirals; +, irregulars.

and moving through the coronal gas of the parent galaxy. The companion has a chance to preserve its gas only when the gravitational binding of this gas with the companion is stronger than the pressure of the coronal wind⁸. The gravitational binding energy can be expressed by the formula

$$U = -G\rho_g M_s R_s^{-1}, \quad (1)$$

where G is the gravitational constant, ρ_g the mean gas density of the companion galaxy, M_s and R_s —the mass and the characteristic radius of the companion. The expression for the pressure of the coronal wind is

$$p = \rho_c V^2, \quad (2)$$

where ρ_c is the density of the coronal gas near the perigalaxy of the companion orbit, $V = (GM(R)R^{-1})^{1/2}$ is its orbital velocity and $M(R)$ is the mass of the parent galaxy inside the companion orbit with the radius R . Since with an increasing distance R the density of the coronal gas decreases, the conditions for preserving the gas are the better the larger is R whereas in the inner regions of the corona we see only elliptical galaxies from which the gas is blown away by the coronal wind.

In order to estimate the mass of the gaseous corona, M_c , we have determined the density of the corona at various distances from the centre of the parent galaxy. This can be done, using the data on galaxies along the line of segregation, where the gravitational binding energy, $|U|$, is approximately equal to the pressure of the coronal wind, p .

According to Roberts⁹ the mass of dwarf irregular and spiral galaxies is proportional to their luminosity and the mean density of stars and gas is constant, and we have $R_s \sim M_s^{1/3}$ and

$|U| \sim M_s^{2/3} \sim L_s^{2/3}$. The segregation line between two morphological types of galaxies is a straight one $L_s \sim R^{-3}$, therefore $|U| \sim R^{-2}$. On the other hand, supposing a power law for the density of the corona

$$\rho_c \sim R^{-\alpha} \quad (3)$$

we obtain $M(R) \sim R^{3-\alpha}$ and $p \sim R^{2(1-\alpha)}$. By comparing both expressions we get $\alpha = 2$.

The dependence $\rho_c \sim R^{-2}$ leads us to the conclusion that outside the optically visible parts of the primary galaxy, where the density distribution is chiefly determined by the corona, the inner mass of the Galaxy, $M(R) \sim R$ and the circular velocity $V^2 \sim M(R)R^{-1} = \text{const}$. This result agrees well with observational data on the circular velocity in the periphery of giant spiral galaxies¹⁰.

The derived density distribution agrees well with our previous results obtained from the dynamics of double galaxies¹. For the distance $R_0 = 15$ kpc we found for the coronal gas density $\rho_0 = 2 \times 10^{-25} \text{ g cm}^{-3}$. To estimate the total mass of the corona, the power law (3) cannot be integrated to infinity since the integral diverges. This shows that at large distances the density must decrease more rapidly than given by the law (3). On the other hand, we conclude from Fig. 1 that this law can be used until $R^0 = 300$ kpc. Using this value of R^0 as a limiting radius of the corona, we obtain for its mass

$$M_c \approx 4\pi\rho_0 R_0^2 R^0 \approx 2 \times 10^{12} \text{ solar masses} \quad (4)$$

in good agreement with our dynamical estimate¹. The mass found should be regarded as a lower limit of the mass of the corona since the adopted limiting radius R^0 may be underestimated.

As noted by Oort¹¹, the presence of large amounts of intergalactic gas is not surprising since the formation of galaxies is generally believed not to have been so efficient as to condense into stars all the diffuse matter in the Universe. Our data suggest that the intergalactic gas is not randomly distributed between galaxies, but is concentrated around massive galaxies, forming their coronas.

Dwarf elliptical companions of our Galaxy have been studied for stellar composition, which is almost identical to the oldest halo population stars⁴. This shows that star formation stopped soon after the formation of these galaxies. The space density of matter in the galaxies considered is small and under normal circumstances gas would be consumed at a very low rate¹². A sudden cessation of star formation can be understood as an effect of the galactic corona, which blew the gas out from the nearby companions at an early stage of their history. But this points to the presence of the corona around the Galaxy just after its formation. At that early time the corona had to be gaseous.

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An attempt to detect the effects of cosmic gamma-ray bursts in the lower ionosphere

DETECTION of gamma-ray bursts of cosmic origin by detectors onboard Vela, OGO, IMP-6 and OSO-7 satellites¹⁻³ was one of the major surprises of observational astronomy recently. The bursts were observed over the energy range 7 keV to 1.5 MeV and were found to have time durations ranging from less than a second to about 80 s with integrated flux density between a few times 10^{-6} and 3×10^{-4} erg cm⁻² for different events. From the 20 events that have been detected so far (from data accumulated over 5 yr) it seems that these events are distributed almost isotropically on the celestial sphere and occur at frequencies of about four to five each year, at the level of sensitivity of Vela satellites. In view of the absence of definitive positive associations with well known transient phenomena¹⁻⁴ such as supernovae, galactic radio noise spikes, rapid atmospheric fluorescence increases, Cygnus X-3 radio flares or even with gravitational radiation events, continuous patrol for detecting these events through as many independent techniques as possible is very important to understand the nature of their origin.

So we have searched for the effects of these bursts on the VLF propagation characteristics in the D-region of the ionosphere; a technique^{5,6} which earlier proved successful for the detection of X rays (~ 1 –10 keV) from sources such as Sco X-1. In short, the method consists of measuring the field strength of VLF propagation that shows absorption corresponding to the transit times of the X-ray emitting celestial bodies because of the resulting enhanced ionisation in the lower ionosphere. The effects of these bursts on the VLF propagation are investigated by examining the data on field strength variations recorded at Ahmedabad corresponding to 164 kHz transmission from Tashkent for several of the events recorded by the Vela satellites⁷. Further, since it is easier to delineate the ionospheric effects due to celestial photons in the absence of solar X rays, only those events that took place between the local times of 1900h and 0700h, corresponding to the geographical coordinates of the reflection point of these 164 kHz waves (32° N, 75° E) were chosen. Table 1 summarises the results of such a search for a number of bursts selected on the above criterion. The column on the observability of the event indicates whether the direction of the sky corresponding to the particular burst was within the visibility of the reflection point. Careful search of the recorded field strength data revealed no observable field strength variations associated with any of the above burst events. Figure 1 shows

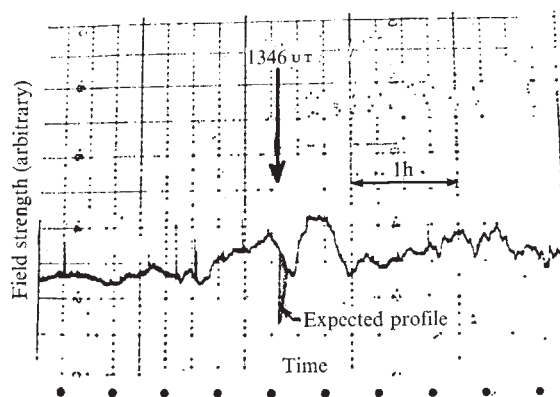


Fig. 1 Photograph of a typical record of the 164 kHz field strength variation recorded at Ahmedabad during March 28, 1972 event. This event had a total duration of ~ 65 s.

the relevant portion of a typical field strength record registered at Ahmedabad corresponding to the time of the occurrence of the event dated March 28, 1971. The dotted line in the figure indicates the expected time profile⁷ of VLF field strength, if the X-ray burst event under consideration had an observable effect on the lower D-region of the ionosphere. The absence of an observable change in the field strength associated with the burst suggests that the X rays from this event did not have any significant influence on the ionisation of the D-region. Assuming that at least half of the uncertain cases should have been favourable from the observational standpoint, we conclude the results of the search for ionospheric effects of these events as negative.

Next, we estimated the electron production rates in the D-region for a typical event such as of May 14, 1972, for which detailed spectral data⁸ are available above 7 keV. Since the atmospheric region between 90 and 70 km is most responsive to X rays between 1–10 keV (refs 6, 9), we have extrapolated the observed⁸ power law number spectrum with an index of ~ 1.4 down to 1 keV for these calculations. Recent observations by Apollo 16 down to 2 keV justify such an extrapolation procedure (L. E. Peterson, private communication). The electron production rates for different heights are estimated for the two intensity peaks observed in the burst by the usual method⁶ using CIRA 1965 model of the atmosphere. The results are shown in Fig. 2 for the case of two zenith angles: $\chi = 0^\circ$ and 40° together with that of Sco X-1 at $\chi = 45^\circ$ for comparison. Even though the electron production rates at the time of the second peak of the burst seem to be comparable with those from Sco X-1, the average production integrated over the duration of the burst of about 50 s is found to be at least a factor of two lower. One striking feature of the production profile is that the altitude at which the maximum rate of ionisation occurs for the burst is around 70 km compared with 85 km for Sco X-1. An

Table 1 Burst search data

Vela event number	Date	UT		Duration (s)	Position coordinates for 3 S/C coincidences				Circle of position for 2 S/C coincidences			Estimated flux (erg cm ²)	Observability of the event from the reflection point	Whether effect is seen
		h	min		Position 1		Position 2		α	δ	Radius (degrees)			
					α	δ	α	δ						
6 9-4	17.10.1969	21	42	1.5					156	1	83	4×10^{-5}	Uncertain	No
70-2	22.8.1970	16	50	10	143	65	205	25	—	—	—	1×10^{-4}	Not visible	No
70-3	1.12.1971	20	01	—					91	-30	16	4×10^{-5}	Visible	No
71-1	2.1.1971	19	11	10	Poor intersatellite timings							1×10^{-4}	Uncertain	No
71-3	18.3.1971	15	28	30	75	-5	100	-60				1×10^{-4}	Uncertain	No
72-1	17.1.1972	17	39	30	104	+9	136	-29				7×10^{-5}	Visible	No
72-2	12.3.1972	15	53	—	277	+1	298	+35				5×10^{-5}	Not visible	No
72-3	28.3.1972	13	46	—					283	22	83	1×10^{-4}	Uncertain	No
72-5	1.11.1972	18	57	—	11	+19	309	-56				7×10^{-6}	Uncertain	No
73-2	10.6.1973			—					25	-36	61	1×10^{-4}	Uncertain	No

α and δ are right ascension and declination, respectively, in degrees.

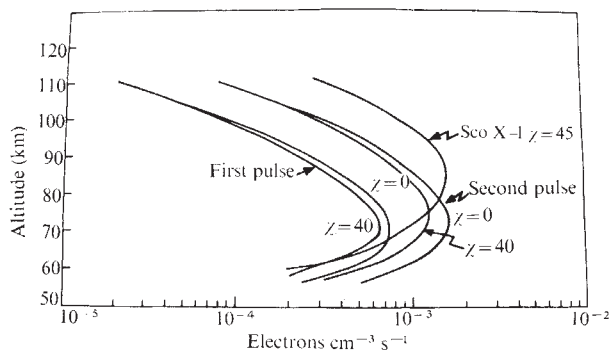


Fig. 2 Electron production rates as a function of altitude for the May 14, 1972, event. Also shown is the production rate due to the X rays from Sco X-1 corresponding to the zenith angle of 45° .

important consequence of such a profile is that the peak electron production rate for these types of bursts yields only low equilibrium electron densities because of the high values of recombination coefficients at the lower altitudes. The value of the recombination coefficient at 70 km is orders of magnitude higher than that at 85 km where X rays (~ 1 –10 keV) from Sco X-1 have maximum influence and where consequently much higher equilibrium electron densities could be produced. Our estimates show that the electron density enhancement arising from this burst is insignificant at 70 km and is much less than 5% over the ambient value at 85 km. Because the burst under consideration is one of the strongest recorded so far, it is clear that the effects to be expected from the events listed in Table 1 should be many factors lower. We therefore conclude that for the type of bursts detected so far, the ionospheric effects are too weak to cause observable perturbations in the VLF propagation.

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No evidence for glacial origin of late Precambrian tilloids in Angola

KRÖNER and Correia¹ claim that the mixtites in the Lower and Upper Tilloid Formations of the West Congo Sequence are “true tillites”, although detailed study supports the idea that they originated as tilloids deposited by submarine mudflows in a geosyncline². I question the validity of their arguments.

Mixtites which enclose striated clasts are not *ipso facto* tillites. Non-glacially striated stones occur in deposits unrelated to glaciation, and glacioclastic detritus may occur in non-glacial deposits. Clasts which have been abraded by non-glacial processes (including tectonism) can resemble glaciated clasts^{3,4}. “Striated pebbles in mudflow deposits . . . have often been noted . . . and their similarity to glacially striated pebbles mentioned”⁴. If Kröner and Correia possess a diagnostic criterion which can provide an objective distinction between glacial and nonglacial striations on boulders, they would do well to state it. If their striated clasts are glacial—even today glaciers exist not far from the sea—that is still not evidence that the mixtites were deposited glacially, as till. (The West Congo geosyncline was at low to equatorial palaeolatitudes during tilloid accumulation⁵.) Glaciated stones in a delta would indicate glaciers in the hinterland but not the glacial deposition of delta sediments or the turbidites issuing from them. The striated boulders occur in a small area². That restriction, which is unlike the situation for Pleistocene and older glacials, argues by itself against the extensive glaciation of land areas. To the north is a vastly larger area which lacks striated stones. Wagner and Wilhelm⁶ examined over a thousand tilloid stones without discovering a single striation. The striated clasts occur near the south-eastern basin margin in Angola; the elevation of the source area may have been sufficient to cause local highland glaciation^{3,7}.

The so-called “dropstones”¹¹ are no such thing and have nothing to do with deposition of the tilloid formations; they are older. It is irrelevant to claim that a formation is glaciogenic because another, underlying, completely unrelated formation contains some small intraformational pebbles (which are not even erratics). Kröner and Correia found these clasts not in any tilloid formation but in the S3de formation (shales, siltstones, quartzites, carbonates and cherts) belonging to the Sansikwa Group. The Sansikwa and Lower Tilloid Formations are separated sharply by a disconformity/unconformity caused

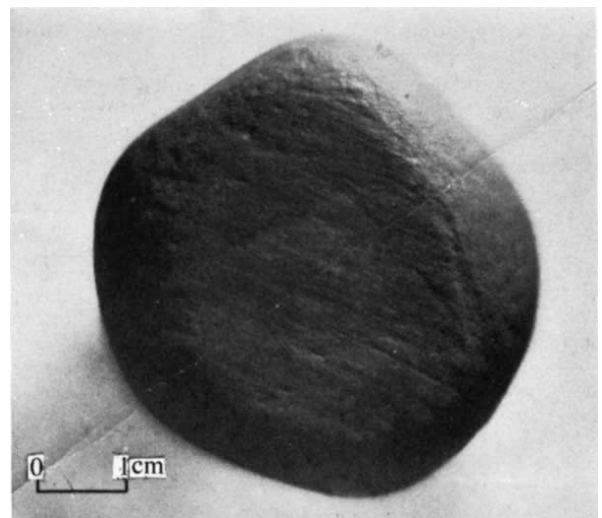


Fig. 1 Small, striated and faceted clast from the lower mixtite (M0), found west of Samba Cajú. The clast is also faceted on the back and these striations there have a markedly different direction from the front face.

by an erosional phase, and the tilloids carry eroded Sansikwa detritus as well as crystalline erratics. No dropstones appear in either the Lower or the Upper Tilloid Formation. The former encloses a thick mudstone level² which, if this formation were glaciogene, could not fail to exhibit ice-rafted erratics. Kröner and Correia's Fig. 2 shows a minute pebble of intraformational siltstone in a subvertical position, only 8 mm long yet strongly deflecting silty laminae both underneath and above it: this is a compaction structure. A silt flake weighing only 0.25 g could not, dropping through water, have bored into bottom silt for a quarter of its length. Nor could it have been implanted subvertically: "in falling through even a few inches of water, pebbles rotate to place their maximum projection area (which is parallel to the long axes) approximately normal to the direction of fall"⁸. True dropstones, when small, lie parallel to the host rock lamination⁹, and are erratics. Nor is the pictured rock varve-like: it is similar to siltstone-shale alternations, such as the Tar Springs Sandstone, in which small silty pods also deflect and penetrate adjacent laminations (ref. 10, Plates 17C and 18C). Such current-transported intraclasts should not be confused with dropstones.

Graded bedding in tilloids and associated sandstones is frequent (though unknown from true tills or aquatills). Mudflows do enclose sandy lenses⁴. Internal stratification is rare, and not common, in tills; Boulton^{11,12} has referred to flow tills which are mudflows.

The argument that turbidity currents could not have deposited the tilloids because they cannot carry coarse material, and that, consequently, these rocks must be glacial, makes no sense because this has never been claimed. Only mudflows have been envisaged, and their carrying capacity is well known^{2,3}.

The tilloid formations are well stratified (the Lower Tilloid contains hundreds of tilloid beds). Their thickness varies from 0–500 m for the Lower Tilloid, and from 5 cm–200 m for the Upper Tilloid. Their greatest lateral extent in Angola is 100 km. Strong facies changes indicate lateral supply, with longitudinal input from geanticlines crossing the basin. That, and the absence of ice-rafting, the erosional bases which cut down 500 m into older strata and other evidence², all argue against glaciomarine deposition.

Tilloid sedimentation was relatively deep marine, evidenced by widespread graded bedding (of normal type, not compositional layering) in tilloids and interbeds, and by the absence of shallow-water structures^{2,3}. The tilloid formations are overlain by hundreds of metres of non-pebbly turbidites, and such thick, extensive, graded sequences do not occur on shelves. They pass up into shallow marine sediments with shallow-water structures. The laminated dolomite caps³ which cover tilloids exhibit the characteristics of deep-water carbonates¹³, lacking shallow-water or lagoonal features. Quartz feldspar sandstone intercalations (with grading) occur in the tilloid formations, as well as among graded greywackes and mudstones in other formations, including flysch. Deep sea investigations show that clean sandstones can occur in a turbidite environment, and they have been reported from flysch troughs^{14–16}.

The association of late Precambrian mixtites with limestone and dolomite is ironclad evidence against a glacial origin³. Experimental investigations and present day occurrences indicate that dolomite precipitation requires a minimum temperature of over 20° C. Recent and ancient precipitated carbonates and evaporites are concentrated at low latitudes¹⁷, where there are warm conditions. The absence of carbonate precipitates among Pleistocene, Permo–Carboniferous, Ordovician and Huronian glacials, confirms this.

Kröner and Correia's objections to nonglacial tilloid deposition are insubstantial; they did not dispute the main lines of evidence based on sedimentology and facies analysis². The mudflow theory, which according to Kröner and Correia was "never accepted by any serious student", emerged from the detailed mapping of 77,000 km² of north-western Angola, covering 40% of the length of the West Congo geosyncline.

The results are available as map sheets with descriptive memoirs published by the Geological and Mining Service of Angola. No other late Precambrian mixtites in Africa, or indeed in the Southern Hemisphere, are as well documented. Serious and unserious students alike are free to interpret the assembled evidence as they will, for much remains to be learnt, but they should consider the whole of the evidence, not disregard essential parts of it.

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DR KRÖNER REPLIES: Schermerhorn concludes that our evidence in favour of a glacial origin for the Angolan mixtites¹ is insubstantial and unsound. During a field excursion in 1973 he failed to convince any of the participants of a mudflow origin for his tilloids. He also rejected a glacial origin for the late Precambrian Numees mixtite in South West Africa^{2–6}. Here I shall correct some of the misinformation he presents.

We have not claimed that mixtites which enclose striated clasts are *ipso facto* tillites, but that the numerous scratched stones in the West Congo 'tilloids' showing striations in various directions, sometimes crossing each other, are additional evidence for glaciogenic deposition of the host rock. Nobody has yet proposed a mechanism by which a small clast (5 cm diameter) can first be faceted and then striated in different directions (Fig. 1) during mudflow deposition, and I believe I have the wide support of students of glaciogenic deposits in identifying such striae as glacial.

Striated clasts are not restricted to a small area but have been found both in Angola and in Zaire¹. It is not surprising that none has yet been reported from northern Angola since outcrops are extremely poor there.

There is no evidence to suggest a low to equatorial palaeolatitude during mixtite deposition in the West Congo basin; neither the precise age of these rocks is known nor has their palaeomagnetism been investigated. Detailed work on the Numees mixtite (a possible equivalent of the lower West Congo mixtite) indicates, however, a high palaeolatitude⁷.

Our small dropstone, which is only one of several found, weighs 11.4 g and not 0.25 g. Schermerhorn's claim that pebbles

drop through water with their flat sides normal to the direction of fall is incorrect and disproved by countless examples of dropstones piercing with their sharp edges or corners through the underlying lamination³⁻⁵. Even small dropstones penetrate the host rock lamination in this fashion; that has been demonstrated to Schermerhorn on a visit to the Numees Formation.

Schermerhorn and Stanton⁸ state that "deposition of the tilloids was thus a type of geosynclinal turbidite sedimentation" and "... a subaqueous mudflow ... even becomes a turbidity current". Also "The beds of quartzite (intercalated in mixtite—A.K.) must have arrived as turbidity currents".

The mixtites extend for more than 800 km in the West Congo Basin and no mechanism can explain this distribution satisfactorily by mudflow deposition.

The association of mixtites with carbonates is characteristic of most late Precambrian glaciogenic deposits⁹ but, contrary to Schermerhorn's belief, it is not evidence against glacial deposition. The Numees tillite³⁻⁵ and the Bthaat Ergil Group in, Mauretania¹⁰, to cite only two well documented examples, are both undisputably related to ancient glaciation but, nevertheless, contain carbonate beds.

Dr Schermerhorn claims boldly but incorrectly that the Angolan mixtites are the best documented of all such sediments in Africa or in the Southern Hemisphere, though only one publication had appeared about them before ours. If it were true, the argument about their origin would long have been settled in favour of glacial deposition.

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Work of fracture of natural cellulose

THE work of fracture (that is, the energy needed in order to form a fracture surface roughly at right angles to the grain direction) is, for timber, exceptionally high in relation to other mechanical properties and density. It compares well, on a weight basis, with that of ductile metals. Our tests on air dry (12% moisture content) pitchpine (*Pseudotsuga taxifolia*) for instance, give a mean value of $0.92 \times 10^4 \text{ J m}^{-2}$. That is far higher than the energy needed to break the interatomic bonds in any given cross section of timber (which can hardly exceed 2 J m^{-2}). It also seems to be noticeably higher than the energy that is likely to be absorbed by the fibre pull-out mechanisms which operate in conventional, artificial fibre composites¹. There is reason therefore to suspect the existence of a special energy absorbing mechanism during fracture in trees and presumably, in other, plants.

Air dry timber has normally a macroscopic breaking strain in tension along the grain of about 1.0%, which is not very different from that of the cellulose fibrillae of which it is principally composed. Such a low breaking strain is not obviously compatible with the absorption of so much energy at fracture.

Cowdrey and Preston² have shown, however, that in the cell walls of timber the cellulose fibrillae are disposed in a preponderantly helical manner, making an angle which may vary between 6° and 30° in different cells but which always has the same sense in any one tree.

Page *et al.*³ have shown that when an individual cellulose cell is detached from its surroundings and pulled in tension, the walls buckle into the lumen in such a way that total longitudinal extensions of the cell of between 15% and 20% are possible. Page, however, was concerned with paper fibres and not with the fracture energy of timber.

We have made large ($\sim 2 \text{ mm}$ diameter) model cellulose fibres by winding glass and carbon fibres into hollow helices with resin. When tested individually in tension, such tubes behave elastically up to a well defined yield point. Beyond that point the tube wall buckles and the equivalent of plastic yield takes place, enabling the tube to extend irreversibly by 10–20%, and thus to absorb a great deal of energy. We have made composite models resembling timber, by glueing together a number of parallel tubes of this type, and these exhibit experimental works of fracture up to about $40 \times 10^4 \text{ J m}^{-2}$, which may be higher than any value previously recorded for a non-metallic material.

We have observed that the fracture behaviour of timber, watched under the optical microscope, much resembles that of our fibre-composite models. The first irreversible event to be observed is the lateral separation of many of the cells in the immediate neighbourhood of the fracture (see ref. 4). That enables the walls of the cells in that region to buckle, and the cells themselves to elongate. Thus, although the elastic strain in the timber as a whole seldom much exceeds 1.0%, the cells close to the fracture surface typically extend 15–20% before breaking, absorbing much energy as they do so.

The adoption of a helical arrangement of reinforcing fibrillae naturally, involves some reduction in the maximum attainable value of longitudinal Young's Modulus. By calculation this reduction seems to be proportional to the square of the cosine of the helical fibre angle. That is equivalent to the loss of about 1% for a 6° helix, increasing to 25% for a 30° helix. Such a loss of stiffness may well be regarded as an acceptable price to pay for the acquisition of so much fracture toughness.

We acknowledge support from the Science Research Council for this research. A patent application is pending.

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Imparting strength and toughness to brittle composites

HIGH strength and high toughness are usually mutually exclusive in composites which have brittle filaments in a brittle matrix. The high tensile strength characteristic of strong interfacial filament-matrix bonding can, however, be combined with the high fracture toughness of weak interfacial bonding if the filaments are arranged to have alternate sections of high and low shear stress (and low and high toughness). Such weak and strong areas can be achieved by appropriate intermittent

coating of the fibres. The strong regions ensure that the filament strength is picked up. Randomly positioned weak areas effectively blunt running cracks by the Cook-Gordon mechanism¹, which in turn produces long pull-out lengths with an associated large contribution to toughness. Boron-epoxy composites of volume fraction 0.20–0.25 have been made in this way; they have fracture toughnesses of over 200 kJ m^{-2} , and they retain rule of mixtures tensile strengths ($\sim 650 \text{ MN m}^{-2}$). At the volume fractions used, that apparently represents K_{IC} values greater than $100 \text{ MN m}^{-3/2}$.

When the interfacial bond between fibre and matrix is strong in composites which have brittle filaments and a brittle matrix, fracture is often caused by rapid matrix cracks which break through all filaments in their paths. The toughness of such composites is low because, in general, the critical transfer length associated with strong interfacial bonding is small, which limits the various components of the total toughness—the 'surfaces' component, Piggott-Fitz-Randolph stress redistribution and Cottrell-Kelly pull-out (see, for example, ref. 2). The critical length is given by $l_{crit} = \sigma_f d / 2\tau$, where σ_f is the filament strength, d the filament diameter, and τ the interfacial shear strength.

A general increase of l_{crit} by lowering the filament-matrix shear bond will increase the toughness, and a relationship between rule of mixtures (ROM) strength, σ , and total composite toughness, R , may be developed by recognising that in, general terms, $R \propto l/\tau$. Weak interfaces throughout the composite, however, reduce the tensile strength quite significantly. The question that presents itself is whether there are means by which the ROM tensile strength can be maintained along with high toughness values.

Marston³ suggested that, providing there were 'sufficient' regions of high interfacial shear stress to ensure that the ROM strength was picked up, the rest of the composite could have quite weak interfacial bonds. Were such a composite to be laid up randomly with respect to weak and strong regions, both high strength and high toughness should be produced simultaneously. Then, if the lengths of the strongly bonded regions were greater than the critical length associated with the strong interfacial τ , the filament strength should be attained. At the same time, those weak interfaces situated randomly ahead of running cracks would serve to blunt the cracks by debonding.

Weakly and strongly bonded interfaces can be achieved by coating intermittently the filaments with some suitable substance before composite lay up. Experiments with silicon vacuum grease (SVG) and polyurethane varnish (PUV) coatings are described here; the uncoated regions have high interfacial shear stress and the coated regions are 'weak'. Tensile strength and toughness specimens were made from layers of intermittently bonded, epoxy composite tape, manufactured on a drum apparatus with a device for coating the filaments before lay up (A.G.A., unpublished). The tape (similar to Avco Rigidite, Prepreg tape) consisted of a $250 \mu\text{m}$ monolayer of B/W filaments in EPON 828 epoxy, backed, for ease of handling, on 760 mm wide nylon scrim cloth about $50 \mu\text{m}$ thick. The tensile strength specimens consisted of 2 layers of the tape, in $100 \text{ mm} \times 6 \text{ mm}$ strips, with end tabs reinforced by additional layers. Most toughness measurements were made on flat sheet edge-crack specimens, similar to American Society for Testing and Materials 'compact tension' specimens in profile. These consisted of 10 layers of tape in panels $76 \text{ mm} \times 76 \text{ mm}$. To prevent the composite arms above the crack from shearing off under load, two outside layers of tape were arranged on each side of the specimens, with the filaments parallel to the crack. The central core of the specimen thus consisted of six unidirectional filaments perpendicular to the starter crack, where, within the limitations of the specimen and tape preparation method, the coated and uncoated layers occurred randomly, relative to each filament. Fracture toughness in the edge-crack specimens was measured for increments of crack area, using Gurney's sector area technique⁴.

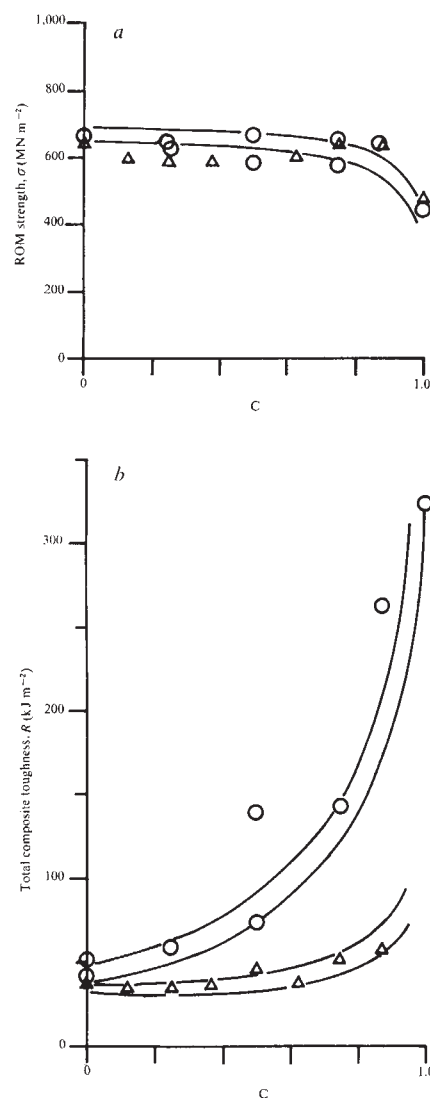


Fig. 1 Tensile strength (a) and edge-crack fracture toughness (b) plotted against the percentage coating, $C = l_c/(l_c + l_{uc})$; where l_c is the coated length, l_{uc} the uncoated length, and $(l_c + l_{uc})$ is the repeat distance of the coating pattern along the filaments. $\circ$, Results with PUV coating; $\triangle$, results with SVG coating.

Tensile strength and edge-crack fracture toughness, for both SVG and PUV coatings, have been plotted against C , the 'percentage coating' (Fig. 1a, b). The tensile data remain at or about the ROM value until C becomes greater than 0.8. In the same range of C , the toughness with SVG coating, shows a modest increase over the initial ($C=0$) case. In contrast, the PUV coatings give marked improvements in toughness when the percentage of coating is increased (Fig. 1a, b): in the edge-crack specimens, with $V_f=0.25$, toughnesses of about 100 kJ m^{-2} are produced for $C=0.5$, and values over 200 kJ m^{-2} occur at large C .

Superimposed on the figures are the predictions of analyses for σ and R , details of which are to be presented elsewhere. Both coating materials maintain tensile strengths of the order of the ROM values up to large C , which suggests that both produce similar coated interfacial shear-strengths. Their effects on toughness are, however, different, which suggests that interfacial shear-strength may not be the controlling parameter for toughness. With PUV it seems that Cook-Gordon debonding takes place (average pull-out lengths of

$I_{crit}/4$ being augmented by additional debonding), but it is apparently absent in the case of SVG.

The Cook-Gordon mechanism itself seems to be only a small toughness sink, but the extra debond lengths markedly increase the pull-out contribution to toughness. The manner in which interfacial properties, other than shear strength, are affected by the coating procedure is therefore both interesting and important, because it is probably the 'toughness' of the interface that is of ultimate concern, rather than the 'strength'. Tensile debonding ahead of a crack, envisaged by Cook and Gordon, is Mode I fracture in the nomenclature of fracture mechanics; the shear debonding implicit in the Outwater-Murphy debonding analysis⁵ for toughness, is in the 'forward sliding' Mode II. Each mode has its own toughness, with explicit relationships with interfacial tensile and shear strengths, which are not obvious.

Further information on the intermittent bond concept is to be presented elsewhere.

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Effect of temperature on recombination luminescence and electron tunnelling

RECOMBINATION luminescence which occurs in alkaline glass after γ irradiation at 77 K, decreases in intensity when the glass is cooled. Between 60 K and 4 K the activation energy is probably about zero. This seems to be direct proof of a tunnelling mechanism.

Electron stabilisation occurs in deep intermolecular traps (2-3 eV) during low-temperature radiolysis of polar glasses¹. After γ irradiation, at 77 K, of aqueous alkaline glasses containing efficient electron scavengers (NO_2^- , NO_3^- , $\text{Fe}(\text{CN})_6^{3-}$), a long range electron transfer occurred, apparently by a tunnelling mechanism^{1,2}. The observation and kinetic study of prolonged isothermal luminescence (ITL) in glassy organic matrices^{3,4} and in alkaline glass³ at 4 K and 77 K, which results from the recombination of trapped electrons (e^-_t) and positive ions, confirms the validity of this mechanism; it also shows that the phenomenon is general in glassy materials.

Our aim was to study the temperature effect on recombination kinetics, using the ITL technique in the range between 77 K and 4 K, where electron tunnelling is apparently the predominant mechanism.

Sodium hydroxide glass (10 mol dm^{-3}) containing phenol (0.03-0.3 mol dm^{-3}) was used because ITL intensity is increased by the presence of phenol³. Irradiations were carried out at 77 K with a cobalt-60 source at a dose rate of 5 krad min^{-1} for periods of 5-15 min. After recording the ITL decay for about 10 min, liquid helium was transferred into the cryostat, whilst monitoring of the luminescence continued.

Following γ irradiation of NaOH glass, whether pure or with phenol, at 4 K or 77 K, ITL can be observed for up to several hours³. The kinetics of the decay, just as in the

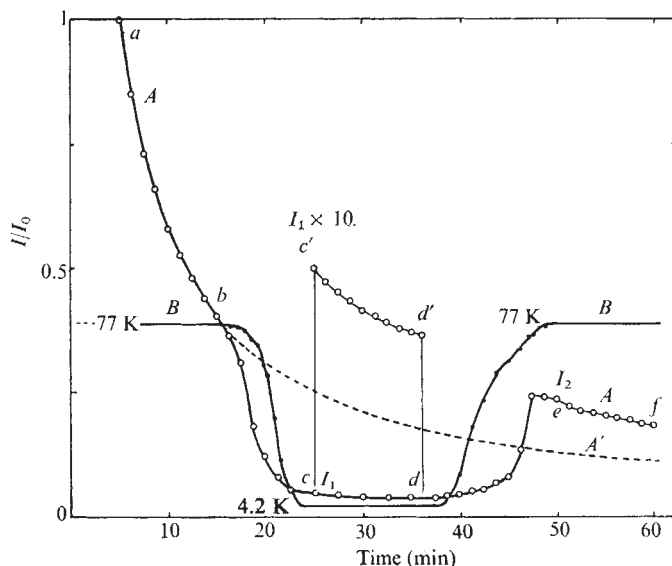


Fig. 1 Recombination luminescence in NaOH glass (10 mol dm^{-3}) containing phenol (0.03 mol dm^{-3}), irradiated with γ rays (dose: 50 krad) at 77 K. *A*, Luminescence intensity (relative units): sections *ab* and *cd*, ITL at 77 K and 4 K (*c'd'* enlarged 10 times); sections *bc* and *de*, luminescence intensity during cooling to 4 K and warm up to 77 K; section *ef*, ITL at 77 K after cooling and warm up cycle. *A'*: extrapolation of the initial ITL decay at 77 K based on the linear plot of $1/I_0$ against time, according to equation (1). *B*, Temperatures corresponding to light intensities of *A*.

case of organic glasses^{4,6}, can be described by the relationship

$$I_0/I = 1 + k(t - t_0) \quad (1)$$

in which I_0 and I are ITL intensities at the beginning of the observation t_0 and at time t ; and k is a coefficient (with the dimension of a rate constant) depending on the duration of γ irradiation, but independent of dose and dose rate. Under identical experimental conditions, the kinetics observed at 4 K and 77 K coincide. The intensity of ITL after irradiation at 4 K is, however, approximately five times larger than after irradiation at 77 K.

Figure 1 shows the change of luminescence intensity with temperature in alkaline glass irradiated at 77 K. The decrease in temperature (Fig. 1, section *bc*) brings about a reduction of luminescence intensity by a factor of about five. Thus, the ITL intensity after irradiation at 4 K is actually about 25 times that after irradiation at 77 K and subsequent cooling to 4 K.

When the temperature is raised again (section *de*), the luminescence intensity increases and reaches a value higher than the normal ITL curve at 77 K (curve *A'*). The kinetics of ITL decay in the sections *ab* (77 K), *cd* (4 K) and *ef* (77 K) can all be represented linearly by equation (1). This indicates that the same recombination mechanism must be predominant under the experimental conditions of these luminescence decays.

This temperature effect is reproducible and can be repeated on the same luminescence decay curve, as long as there is sufficient light intensity.

Because the luminescence intensity at any moment is proportional to the reaction rate of recombination, it can be considered to be proportional to the rate constant when temperature is the only parameter which changes. Therefore, the fivefold decrease in luminescence intensity observed during cooling from 77 K to 4 K suggests an exceedingly low activation energy. In fact, there is practically no change between 60 K and 4 K.

From our thermoluminescence experiments (unpublished), the activation energy for charge recombination, calculated by the initial rise method⁷ for the glow peak at 92 K, was found to be ≈ 7 kcal mol⁻¹ (≈ 0.3 eV). This is an activation energy for a thermal detrapping process which is predominant at the glow peak. Its contribution decreases with decreasing temperature, and therefore the apparent activation energy calculated from the Arrhenius equation gradually decreases until it is practically zero below 60 K, when tunnelling seems to be the only process. Zero activation energy seems to be direct proof of a tunnelling mechanism in the decay of trapped electrons at sufficiently low temperatures.

These results confirm the general rules concerning the contribution of tunnelling to the rate constant of a chemical reaction⁸. They show that below a certain temperature tunnelling plays the predominant role.

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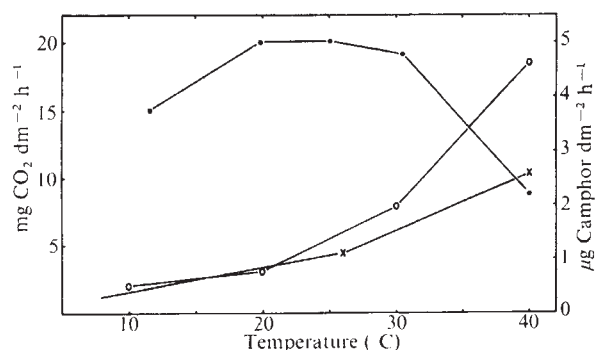


Fig. 1 Temperature curves for photosynthesis under saturating light (●), dark respiration (×) and camphor volatilisation (○) in *S. mellifera*.

A cryogenic pump maintained flow through the loops (1 l min⁻¹ for 10 or 20 min). The organic volatiles were separated from the water which accompanied them and analysed qualitatively by gas chromatography-mass spectroscopy⁵. Gas chromatography alone was used for quantitative analyses. For convenience all quantitative measurements were based on camphor volatilised. The only other major volatile component was 1,8-cineol (23%), levels of which correlated closely with those of camphor (50%). Other terpene hydrocarbons (α -pinene, 8%; β -pinene, 4%; camphene, 4%; limonene, 3%; cymene, 2%; myrcene, 2%; β -phellandrene, 2% and γ -terpinene, 2%), were difficult to quantitate and were not measured.

Steady-state rates of terpene (camphor) volatilisation in the light and dark were determined by placing the plant in the chamber at constant temperature and humidity and monitoring the camphor produced until the rate of volatilisation was constant. Rates were directly proportional to leaf temperature (Fig. 1), and were the same in both light and dark.

Daily carbon gain and terpene loss by *S. mellifera* can be calculated using the temperature curve for the steady-state rate of terpene volatilisation and the gas exchange characteristics (Fig. 1) and the natural daily temperature regime of an area where the plant is dominant⁶. Figure 2 gives the temperature regime and calculated levels of terpene loss and gas exchange for April 1972 at a coastal site (Camp Pendleton, California) where *S. mellifera* is dominant. During this period the plant was physiologically active. The calculated rate of terpene volatilisation was 13.3 µg dm⁻² d⁻¹.

Volatilisation of terpenes from *Salvia mellifera*

We have found that branches of *Salvia mellifera*, an aromatic shrub that grows in coastal southern California, produce sufficient quantities of terpenes to make a significant contribution to the atmospheric content of organic compounds. Our simultaneous qualitative and quantitative analyses of compounds volatilised by intact plants in controlled conditions represent an advance on previous measurements of organic compounds in the atmosphere surrounding plants¹⁻³.

Salvia mellifera, the California black sage, is a shrub (1-2 m tall) distributed throughout the coastal regions of California from the San Francisco Bay area southward. In some immediate coastal regions it is the dominant plant species. Its herbaceous leaves, which are covered with glandular, terpene-containing trichomes, usually drop by the end of the long summer drought.

Net photosynthesis (under saturating light) and dark respiration were measured in an intact branch of a potted plant using a gas analysis system like that previously described⁴. The sample was contained within a cuvette where temperature and humidity were controlled. Photosynthetic and respiration rates were determined for various temperatures (Fig. 1).

The volatile constituents of the air emerging from the gas exchange cuvette were trapped in stainless steel loops which were packed with Pyrex wool and immersed in liquid nitrogen.

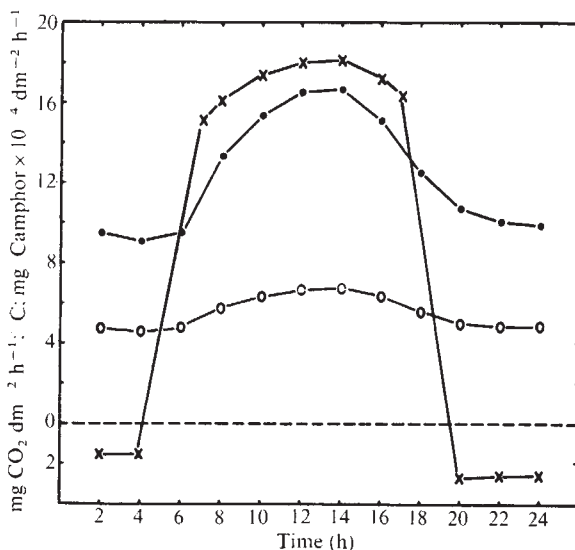


Fig. 2 Mean hourly temperatures for April 1972 at Camp Pendleton (●) and calculated rates of CO₂ exchange assuming saturating light during the light period (×) and camphor volatilisation (○) for *S. mellifera* at that site.

If 5.2 g of CO_2 is required to produce 1 g of camphor (18 mol $\text{CO}_2 \rightarrow 6$ mol 3-PGA $\rightarrow 6$ mol acetyl-CoA $\rightarrow 2$ mol mevalonic acid $\rightarrow 1$ mol camphor), the loss of photosynthate through camphor volatilisation was 0.03% in April. Since camphor represents 50% of the terpene pool in *S. mellifera*, the total loss of carbon by terpene volatilisation was 0.06% during April. This value is about two orders of magnitude below Went's predicted 5% loss of photosynthate as volatile terpenes from *Artemisia tridentata*¹.

S. mellifera comprises 45.7% of the vegetation cover at the Camp Pendleton site. Based on the rate of volatilisation for April and a leaf area index of 2.6, 3.1 kg terpene $\text{km}^{-2} \text{d}^{-1}$ would have been released into the atmosphere from this site in April. This rate is one order of magnitude below that predicted for *A. tridentata* (50 kg $\text{km}^{-2} \text{d}^{-1}$) (ref. 1).

The measured rates of terpene volatilisation from intact branches of *S. mellifera*, which is highly aromatic, do not account for the high levels of atmospheric organics measured by Rasmussen and Went² in the field. Their observations that levels increased immediately after fields were mowed and at times of maximum leaf drop in deciduous forests, but not with the appearance of new foliage in the spring, suggest that the source of volatiles was primarily decomposition or loss from dead cells, rather than volatilisation from intact plant material. Detailed analyses of field samples are required to determine the nature of volatile organics in the atmosphere; contrary to general assumption, many may not be terpenoid³.

Our study demonstrates significant terpene volatilisation from healthy, intact leaf material of *S. mellifera*. This loss of carbon represents a substantial metabolic cost which implies that it confers some benefit on the plant. Terpenes, especially camphor and 1,8-cineol, from *S. mellifera* have been suggested to act as phytotoxins in the ecosystem⁷. Quantitative data such as ours will allow a more comprehensive analysis of this proposal by indicating the contribution of *Salvia* populations to natural levels of terpenes in the ecosystem—levels at which the compounds must be active.

The terpene content of the leaves, which was measured as 31.5 mg dm^{-2} by steam distillation, represents a large amount of potentially volatile material, which may lead to intervals of high atmospheric terpene levels at the time of leaf senescence. This might be especially important in the coastal sage communities of California, where leaf drop and high summer temperatures occur simultaneously.

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Lead levels in the water of suburban Glasgow

THE association of soft water with increased plumbosolvency from domestic cold water systems¹ has been shown by Beattie *et al.*² to result in elevated levels of blood lead in persons resident in lead plumbed houses in soft water areas. This effect was particularly marked in people resident in houses supplied with cold water from lead lined storage tanks. Here we report a study carried out in an area of Glasgow whose residents were principally owner-occupiers of social classes one and two.

The houses studied were identified from the intersections of a grid placed over a 1 in 2,500 scale Ordnance Survey map. Each house was numbered and selected from a table of random numbers. This gave a total of 120 residences. After selection, householders were invited to take part in this survey of the lead status of their homes. The person first contacted was usually the person studied from each household. No person was included in the survey who had been resident in the house for less than 1 yr. Each participant had 10 ml venous blood withdrawn, heparinised and stored in ice. At the same time 250 ml bottles were left for a collection of the first morning flush of water from the cold water system.

Erythrocyte δ -aminolaevulinic acid dehydratase (EC 4, 2, 1, 24) (ALA.D) the second enzyme of the haem biosynthetic pathway, which is often taken as a bioanalytical measure of lead exposure, was measured by the Commission of the European Communities Standard Method³. The results were expressed as μmol ALA utilised per ml RBC per min (Units). Blood and water lead levels were measured by flameless atomic absorption spectrophotometry (Table 1).

In the assessment of the results, the houses were arbitrarily divided into two groups; those less than 20 yr old (group A) and those greater than 20 yr old (group B). This division was chosen because before 1939 these houses were constructed with lead plumbing, and until 1967 lead 'link' pipes were used to connect the domestic copper system to the cast iron main. There were 50 houses in the survey, 12 in group A and 38 in group B. On the basis of this division, it was found that there was a highly significant difference in the water lead content of the two groups. The older group of houses (group B) had a mean water lead content of about 350 $\mu\text{g l}^{-1}$, that is, ten times greater than the mean of 35 $\mu\text{g l}^{-1}$ in group A. Indeed, in the latter group no water lead value was greater than 100 $\mu\text{g l}^{-1}$ (WHO upper acceptable limit), and 82% of the houses in group B were above this limit. Associated with this increase in water lead levels there was a significant 1.5-fold elevation of blood lead in the occupants of the older houses. There were, however, no significant differences in the age of resident examined, the duration of residence or of the sex ratio between the groups. When the water lead (PbW) and blood lead (PbB) levels were compared for all subjects in the study, a significant linear regression was found with regression coefficient $r = 0.417$ using the equation $\text{PbB} = 0.018\text{PbW} + 22.9$.

Table 1 Lead in Glasgow water

	Group A (< 20 yr)	Group B (> 20 yr)	Significance
No. in group	12	38	
Mean age of house	13.5 $\pm$ 4.4	42.6 $\pm$ 4.2	—
Time of residence	8.8 $\pm$ 5.3	16.1 $\pm$ 12.5	NS*
Age of resident	41.3 $\pm$ 18.8	46.7 $\pm$ 17.8	NS
Sex ratio (female: male)	2 : 1	29 : 9	—
Water lead ($\mu\text{g l}^{-1}$)	34.6 $\pm$ 29.5	353.1 $\pm$ 255.6	$P < 0.001$
Blood lead ($\mu\text{g per 100 ml}$)	19.8 $\pm$ 8.4	30.5 $\pm$ 10.6	$P < 0.01$
δ -aminolaevulinic acid dehydratase (units)	27.8 $\pm$ 13.2	22.3 $\pm$ 10.4	NS
ALA.D			
Residence > 5 yr (No. of subjects)	30.4 $\pm$ 12.9 (10)	18.9 $\pm$ 5.1 (30)	$P < 0.005$

* Not significant

In the initial analysis, no significant difference was found between the groups for ALA.D activity. When subjects who had been resident in the houses for less than 5 yr were removed from the analysis, however, resulting in 10 subjects in group A houses and 30 in group B houses, a highly significant difference in ALA.D activities was observed. The difference was of the same order as the difference in blood lead values (1.5 times). As has been often previously shown, there was a highly significant negative exponential regression relationship between the blood lead and blood ALA.D with $r = -0.46$ using the equation $ALA.D = 34.5e^{-0.017 PbB}$.

Of perhaps greater interest was the observation that for all residents in the survey there was a significant negative exponential regression relationship between erythrocyte ALA.D activities and water lead levels, using the equation $ALA.D (=) 25.7e^{-0.0006 PbW}$.

These results clearly demonstrate the possible environmental hazard from lead plumbing, especially in a soft water area such as Glasgow. It is difficult, indeed impossible, to determine the point at which low levels of lead ingestion may harm exposed persons. It is, however, significant that biochemical changes can be observed in ALA.D at blood lead levels as low as those found in this survey which represents the normal levels found in a large urban and suburban population. Remedial measures should, and indeed are, being taken in such areas of possible hazard, including the removal of lead plumbing and the 'hardening' of water with calcium and magnesium salts to lower the plumbosolvency of the water.

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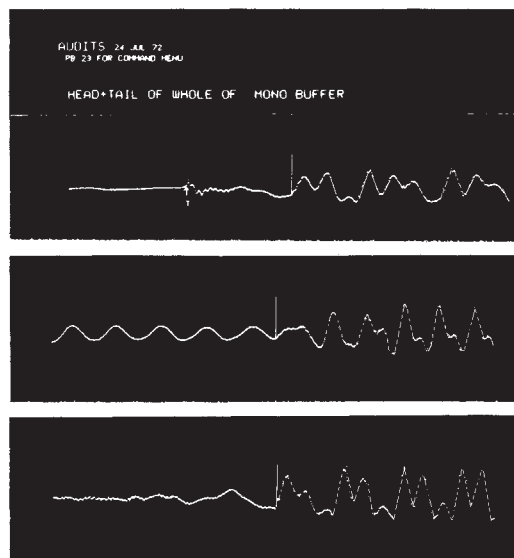


Fig. 1 Sample oscillographic displays of the utterances, illustrating the method used to analyse VOTs. The top display shows the initial segment of a [bi] utterance containing a short-lag VOT (voicing onset occurs shortly after the release burst of the consonant). The pointer T indicates the onset of the release burst, the vertical line to the right of T marks the onset of voicing, the time difference between the T pointer and the vertical line is displayed at the bottom right of the oscilloscope screen. For this utterance, the VOT is measured to be +11.6 ms. The middle display shows a segment of a prevoiced [bi] syllable (voicing onset precedes the release burst of the consonant). For prevoiced [bi]s, the pointer T (not shown in the display segment here) is set at the onset of voicing, while the vertical line cursor is aligned with the onset of the release burst. The time difference between the two markers is taken as the measure of VOT, in this case –86.6 ms (negative VOT values signify prevoicing). The bottom display shows a segment of aspiration and the onset of voicing for a [pi] utterance. The T pointer is set at the onset of the release burst (not shown in this display segment), and the vertical line cursor is moved to the position of voicing onset as in the case of the short-lag VOT [bi] utterances. The actual screen size is 14 × 13 inches, and the vertical and horizontal display scales can be magnified to facilitate the marking procedure. The displays are taken from ref. 13.

Feature processing in the perception and production of speech

It has long been argued that the perception and production of human speech are mediated by a common mechanism^{1–3}; nonetheless, convincing evidence has not yet been obtained in favour of this view⁴. We discuss here, however, a series of experiments in perceptuo-motor adaptation that provide fairly direct evidence favouring the existence of such a mechanism. Our findings indicate that this mechanism processes information about individual phonetic features of consonants^{5–7}, rather than processing these consonants as integral units.

Subjects were instructed to utter a given consonant + vowel syllable immediately after listening to repetitions of a selected adapting syllable. The utterances were analysed to determine whether a feature of the waveforms systematically varied as a function of perceptual adaptation. We concentrated on a single phonetic feature—voicing, in word-initial stop consonants. In English, voicing minimally distinguishes voiced from voiceless stop consonants—[b] from [p], [d] from [t], and [g] from [k]. This feature was chosen because: (a) it has an acoustic correlate that can be readily measured from real speech waveforms, (b) numerous data on perceptual adaptation have been obtained for this feature^{8–11}, and (c) the feature serves to mark phonemic distinctions in virtually all natural languages¹².

During speech production, voicing distinctions for word-initial stop consonants can be signalled by voice onset time (VOT), a temporal relation between the release of oral closure and the onset of vocal cord vibration. Voiceless stops ([p], [t], and [k]) are normally produced with an onset of vocal cord vibration that lags behind the release of closure by more than 30 ms, whereas voiced stops ([b], [d], and [g]) are produced with an earlier onset of voicing. In this research the question of perceptuo-motor control was studied by analysing VOT values for a sample of approximately 5,000 consonant + vowel utterances, articulated under different conditions of perceptual adaptation.

Throughout the tests, three adapting syllables were used—[i], [pi], and [bi]. These syllables were five-formant synthetic speech patterns generated on a terminal analogue speech synthesiser (acoustic properties of these syllables are described elsewhere¹³). Subjects were tested individually in a sound-insulated chamber. Forty adaptation trials were administered in blocks of 10 during an hour-long session, with a 5-min rest period between blocks. On each trial in a given block, the subjects were presented with 70 repetitions of a single adapting stimulus, with an inter-repetition interval of 350 ms. Subjects listened to the adapting syllable binaurally over headphones. They were instructed to hold their tongues firmly between their teeth and lips while listening and were told to minimise subvocalisation. After 70 repetitions of the adapting syllable were presented, subjects uttered a single CV syllable in a natural voice, as soon as possible (mean response latency < 1.5 s).

Each utterance was analysed oscillographically, aided by the AUDITS computer program¹⁴. A cursor was manipulated to mark the onset of the consonant release burst and the onset of voicing, and the time difference between the two marks was displayed on the oscilloscope screen to the nearest 100 μ s. The accuracy of each VOT measurement was estimated to be within ± 1 ms, except in the case of voiced consonants with VOTs near 0 ms, where the accuracy was reduced to about ± 3 ms. In all cases, accuracy was limited by our ability to specify the onset of voicing. Examples of the oscilloscope displays are shown in Fig. 1.

In the first experiments¹³, subjects were instructed to utter the syllable [pi] or [bi] on each block of trials after repetitive listening to [i], [pi], or [bi]. The results for the [pi] utterances showed a significant decline in VOT values after perceptual adaptation to [pi], compared with the VOT values obtained after adaptation to the isolated vowel [i] ($P < 0.05$). Thirteen of the 16 subjects showed this decline in VOT as a function of perceptual adaptation; the mean VOT shift was 5.6 ms. For the [bi] utterances, no systematic shift in VOT was obtained after perceptual adaptation to [bi], compared with VOTs obtained after adaptation to [i].

The asymmetry observed between the effects for [pi] and [bi] utterances was in accord with a number of additional facts. Whereas the distribution of the VOT values for the [pi] utterances was approximately Gaussian under all test conditions, and showed no highly elevated response peak, the distribution for the [bi] utterances was non-Gaussian and showed a very strong clustering of responses within the narrow range between 0 and 20 ms VOT. The presence of a well-defined target region for the VOT production of the [bi] utterances—a consequence of simple mechanical coupling by airflow¹⁵—may have accounted for their resistance to adaptation. Converging evidence of asymmetry between the processing of voiced and voiceless stops has been obtained in studies of perceptual adaptation^{8,9}, speech production^{12,16,17} and neonate perception¹⁸.

We were able to conclude that the perceptuo-motor adaptation effect for [pi] represented the fatiguing of a mechanism that mediates an aspect of both speech perception and production. In reaching this conclusion, we ruled out two alternative interpretations—perceptuo-motor compensation¹⁹ and voluntary mimicry of the adapting syllable (see ref. 13 for details). Having demonstrated the existence of a perceptuo-motor effect, we conducted a second series of experiments to begin to determine the nature of the mechanism tapped by this procedure. We wanted to find out in particular whether the mechanism processes information about the stop consonants *in toto* or whether it

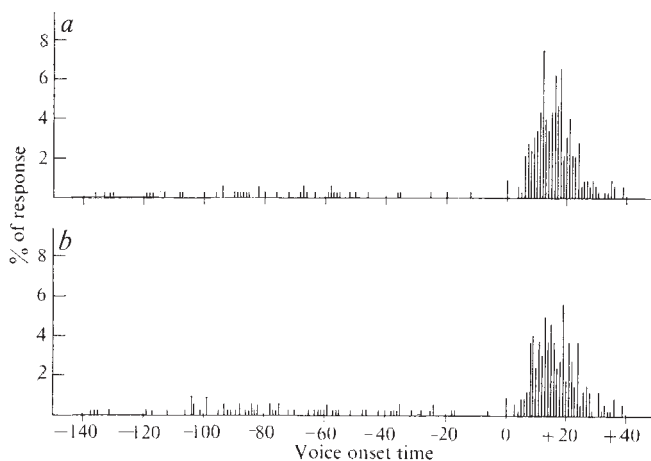


Fig. 3 Frequency distribution of the [di] utterances for the group of 32 subjects after perceptual adaption to [i] (a) [bi] (b).

processes information about the individual phonetic features that comprise these consonants⁵⁻⁷.

In linguistic theory, the notion that consonants can be represented as bundles of distinctive features—features such as 'voicing' and 'place of articulation', to a first approximation—provides the chief means of accounting for relationships of phonetic similarity. Previous studies of perceptual adaptation^{8,9} provided evidence that linguistic features may be processed during some stage of speech perception. The present aim was to determine whether these features are extracted at the level of processing which mediates both speech perception and production. We studied this question by examining the case in which both the adapting syllable and the selected utterance shared the relevant voicing property, but differed from each other in place of articulation.

In these experiments, subjects were presented the adapting stimuli [i], [pi] and [bi] as before. But now, the subjects' task was to utter the syllable [ti] or [di] after each group of 70 adapting repetitions. For each of the two CV utterance types, perceptual adaptation to [i] was counterbalanced across blocks of ten trials with perceptual adaptation to the CV syllable belonging to the same voicing category as the utterance.

The results of the experiments appear in Figs 2 and 3, where the VOT frequency distribution of the [ti] and [di] utterances are shown for each test condition. A total of 1,280 utterances was examined in these experiments (320 in each of the four experimental conditions). For [ti] utterances, a systematic decline in VOT was obtained after adaptation to [pi], compared with the VOT values obtained after adaptation with the isolated vowel ($P < 0.01$). Twenty-three of the 32 subjects showed this effect; the mean magnitude of effect was 3.2 ms. This average magnitude was about 2% larger than that obtained for [pi] utterances with the same subjects (based on data presented in ref. 13 and additional tests). The obtained effect for the voiceless stops thus seems to be entirely feature-specific. No systematic shift was obtained in the VOT values of the [di] utterances after adaptation to [bi]. The asymmetry observed between the effects for [ti] and [di] utterances corresponded to the asymmetry obtained earlier for [pi] and [bi] utterances. In both cases, perceptuo-motor effects were found for the voiceless stop consonants but not for their voiced counterparts. The corresponding difference in the distributional patterns for VOT held as well for the present data (see Figs 2 and 3).

The results for the [ti] utterances indicate that the stage of processing subserving both the perceptual and motor systems of speech performs at least one highly specialised function—namely, processing information about the voicing property of the consonant. Since the [pi] adapting syllable contained a consonant with a different place of articulation from the [ti] utterance, the perceptuo-motor effect cannot be attributed to a mechanism that processes consonants as indivisible units.

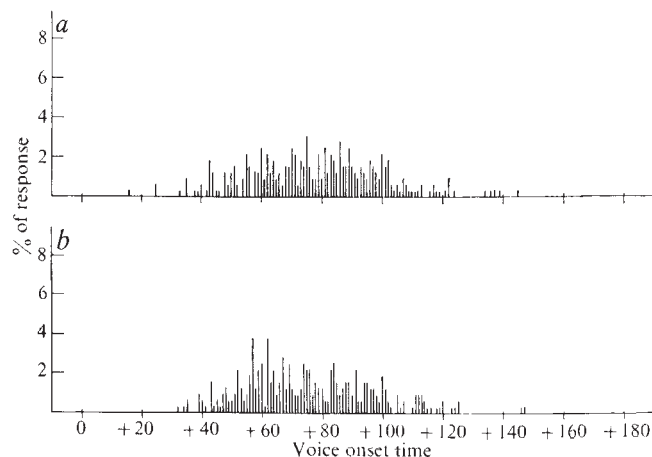


Fig. 2 Frequency distribution of the [ti] utterances for the group of 32 subjects after perceptual adaption to [i] (a) or [pi] (b). This display provides a good record of the range and distributional pattern of VOT values, although it does not easily reveal the small but significant shift in VOTs between the two test conditions.

With the present experimental technique, in combination with direct study of certain laryngeal factors²⁰⁻²², we hope to further define properties of this perceptuo-motor component of the speech system.

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Recognition of mother's voice in early infancy

CERTAIN characteristics of the human voice at normal levels have a great influence on the neonate¹, and infants 1 month old can detect fine differences in speech-like sounds^{2,3}. This enables a selective response to take place when the child meets adults. From birth, babies will turn towards the source of a sound, and this orientation to a voice helps them to learn about faces. We have also observed that infants are more interested in their mother's face when she is talking.

A mother's voice is a frequent and relatively unchanging event in the young infant's auditory world, and its early

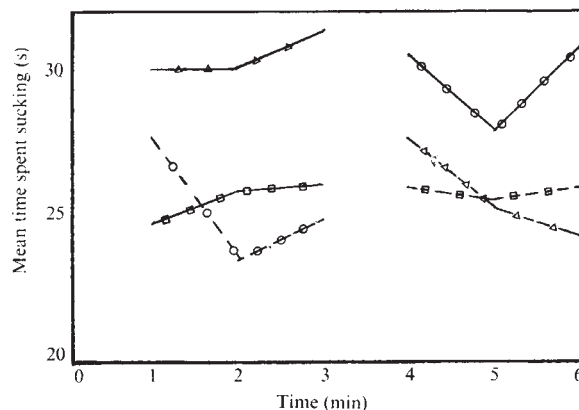


Fig. 1 Contrast effect for voice of mother (—) and stranger (---) measured in mean time per minute spent sucking. Δ , Group I; $\circ$, group II; $\square$, group III (non-contingent). Interaction of voices and group scheduling was significant ($P < 0.05$) as confirmed by analysis of variance, with repeated measure.

recognition has important consequences for the developing bond between mother and child. We report here a study which is the first designed to discover whether normal, 3-week-old infants from intact families can recognise their mother's voice. Successful recognition, we believe, is likely to aid communication.

We allowed the infants to control the auditory feedback they received (for instance, a mother's voice) by sucking on a teat to turn on the auditory stimulus. This technique, successfully employed by Siqueland *et al.*⁴ and Bruner⁵, may be used to find out what infants prefer to hear. (Operant conditioning of non-nutritive sucking has been demonstrated in neonates by Stern *et al.*⁶.) If a change in the way the infant sucks is shown to be dependent on whether it can hear the mother's voice or that of a stranger, it would indicate recognition of the mother's voice.

Babies 20-30 d old, and midway between feeds, were placed, when alert (Pechtl's Stage IV⁷), in an infant seat behind a screen. A blind teat placed in the mouth connected to a pressure transducer, and a light behind a transparent reading panel turned on when the infant sucked normally and alerted the mother to read. (A normal suck was 5 mm of water pressure.) Each baby heard mother's voice live and that of a female stranger equated for loudness, but saw no one. Strangers varied from baby to baby to avoid responses being due to the characteristics of one particular voice.

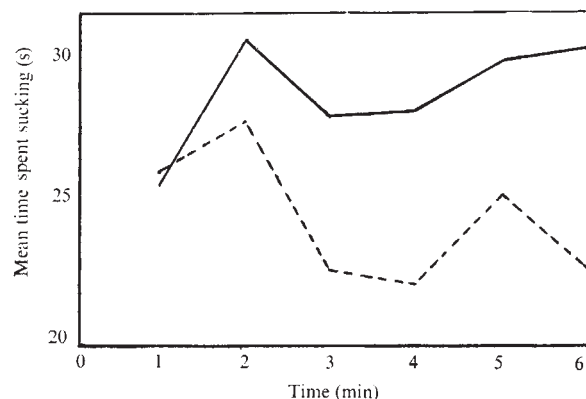


Fig. 2 Mean time spent sucking per minute during training for the contingent groups 1 and 2 combined (—) and non-contingent group (---). The groups and minutes interaction as confirmed by analysis of variance was significant ($P < 0.05$) as was the linear component of the interaction trend analysis ($P < 0.01$).

Table 1 Babies discrimination between voices

Schedule	Contingency training		Voice discrimination	
Group 1 (<i>n</i> = 16 mean age 24 d, s.d. 2.6)	Base line sucking only	First stranger's voice contingent on sucking	Mother's voice contingent on sucking	Second stranger's voice contingent on sucking
Group 2 (<i>n</i> = 16, mean age 24 d, s.d. 2.6)	Base line sucking only	First stranger's voice contingent on sucking	Second stranger's voice contingent on sucking	Mother's voice contingent on sucking
Group 3 (<i>n</i> = 16, mean age 24 d, s.d. 2.2)	Base line sucking only	First stranger's voice controlled by non-contingent schedule	Mother's voice controlled by non-contingent schedule	Second stranger's voice controlled by non-contingent schedule
Time (min)	0-1	2-7 (inclusive)	8-10 (inclusive)	11-13 (inclusive)

A non-contingent control with the same order of voice presentation as group 1 was achieved by equating total duration of feedback experienced by babies in group 1 on an individual subject basis. Schedules were generated by a computer program from the group data.

The procedure began with 1 min of sucking (baseline), and a 6 min training period followed during which the infant had a prolonged opportunity to learn the contingency between sucking and voice, and to integrate response and feedback. Data on discrimination between voices came from two further 3 min periods during which sucking produced the mother's and then the stranger's voice or vice versa (see Table 1).

The results for the voice presentation show that time spent sucking per minute and number of sucks per minute were greater ($P < 0.001$ in both cases) when mother's voice was contingent on sucking. The duration of sucking for all groups is plotted in Fig. 1. No difference was recorded in the non-contingent group. Thus, the possibility of mother's voice increasing the arousal level of the infant would not account for the reported increase in sucking activity since the feedback-matching control effectively differentiates between stimulus and operant control of behaviour. Great variability in the duration of bursts of sucking in the first minute that mother's voice was available is consistent with the view that the mother's voice was initially responded to in the control group.

Non-nutritive or 'comfort' sucking typically has a rhythmic pattern in which bursts of sucking are regularly interspersed with pauses in activity. Babies could have achieved the increase in total time spent sucking when the mother's voice was available by a number of different strategies. In the event, for the mother's voice, mean burst length increased ($P < 0.025$) and mean pause length shortened ($P < 0.01$) as confirmed by analysis of variance. It is interesting that regulation of the sucking pattern was both systematic across contingent groups and an appropriate modification of behaviour if the mother's voice was to be found attractive.

Effects under contingent and non-contingent scheduling during the training period are plotted in Fig 2. Analysis indicates that infants were learning to produce a reward—that of a complex auditory feedback as a result of their behaviour. Only future investigation can establish whether it is sensitivity to the contingencies inherent in the present task, or the processing of correlated information that is being demonstrated by these young infants.

Babies will expend greater effort, it seems, to hear a familiar voice. Our findings suggest that infants have already learnt some characteristics of their mother's voice, although we are not in a position to say which aspect they may be recognising. Nevertheless, successful discrimination of mother's voice is occurring before they are a month old.

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Pathogenicity and cerato-ulmin production in *Ceratocystis ulmi*

THE recognition of aggressive strains of *Ceratocystis ulmi* associated with the epidemic of Dutch elm disease in Britain directed attention to the importance of variation in the pathogenicity of the causal fungus^{1,2}. British workers^{3,4} have provided evidence of a correlation between pathogenicity and certain cultural characters such as colony appearance and growth rate. Here I describe a correlation between pathogenicity of certain strains of *C. ulmi* and the production of a metabolite named cerato-ulmin which, when introduced to elm seedlings, is capable of producing many of the symptoms of the disease.

Cerato-ulmin was previously described as microstructures⁵ which are produced abundantly in liquid shake cultures of *C. ulmi*. It has unusual characteristics. As already reported^{5,6} these

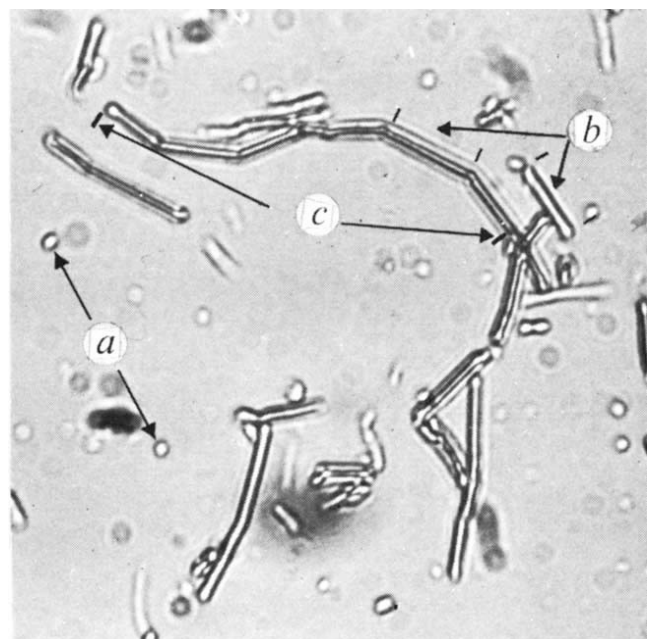


Fig. 1 Three unstable forms of cerato-ulmin in water as observed under the microscope. *a*, 'Unit' (a primary form); *b*, 'rod'; *c*, 'fibril' ($\times 256$). ('Rod' and 'fibril' are assemblages of 'units'.)

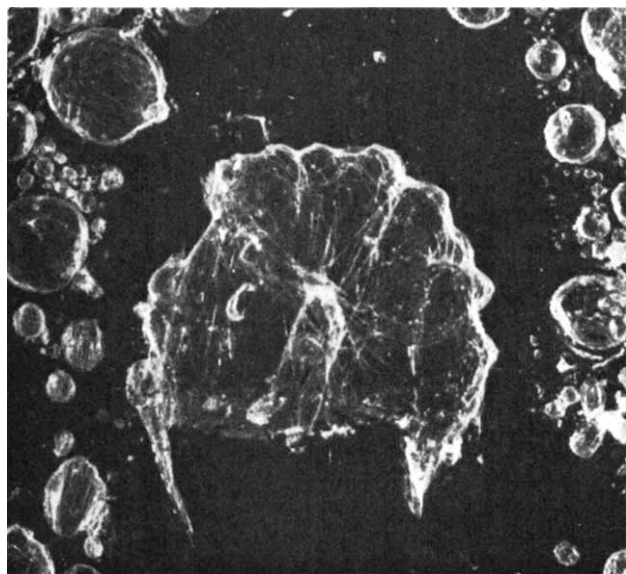


Fig. 2 By bubbling⁶ or vacuum effervescing (S.T., unpublished) rods and fibrils are forced on to the surface of the liquid as bubbles. When these accumulated bubbles burst, circular pieces of membrane, as shown here, remain floating on the liquid surface ($\times 30$).

microstructures are of unstable form, and readily change from 'units' to 'rods' and 'fibrils' (Fig. 1). As 'units' the solution remains clear, but once 'rods' and 'fibrils' are formed the solution becomes milky in appearance. An additional assemblage of cerato-ulmin is 'membrane' (Fig. 2). After centrifugation, however, the fluid becomes clear. Such changes in morphology of cerato-ulmin in water indicate that it may be a liquid crystal, specifically a lyotropic liquid crystal⁷. Cerato-ulmin seems to possess both carbohydrate and peptide moieties in its molecule.

Cerato-ulmin is certainly released into the culture medium but the actual production site has not yet been confirmed. When crushed spores which were repeatedly washed with water to remove cerato-ulmin from the spore surface were extracted, however, no cerato-ulmin has been detected in the extract. It is therefore assumed that cerato-ulmin is produced extracellularly.

When five *Ceratocystis* species other than *C. ulmi* were cultured in the same conditions as those in which *C. ulmi* was grown to produce cerato-ulmin, no cerato-ulmin was detected. These species were *C. dryocoetidis* Kendr. and Moln., *C. fagacearum* (Bretz) Hunt, *C. major* (von Beyma) C. Moreau, *C. minor* (Hedgc.) Hunt and *C. piceae* (Münch) Bakshi. Thus, cerato-ulmin seems to be a specific metabolite for *C. ulmi*.

Cerato-ulmin began to appear in liquid shake culture 3 d after inoculation, while active spore production was continuing and eventually reached 70% of the maximum level. Subsequently, the production of cerato-ulmin was rapidly accelerated but this accelerated production ceased 13 d after inoculation. Spore production was fluctuating at this time following peak production 5 d after inoculation. Beyond 13 d cerato-ulmin remained constant in spite of a levelling off of the spore number. This indicates that cerato-ulmin is a metabolite during active fungal development. In addition, even when a sharp reduction in spore numbers in the culture—probably indicative of autolysis—took place 22 d after inoculation, no change was detected in the level of cerato-ulmin. So cerato-ulmin is not considered an autolytic product of the fungus.

Cerato-ulmin causes white elm (*Ulmus americana* L.) seedlings to display symptoms similar to those produced by Dutch elm disease. A cerato-ulmin aqueous solution (120 mg ml⁻¹) was continuously pressure injected (351.5 g cm⁻²) into the cuttings of 3-yr-old white elm seedlings in greenhouse conditions. An initiation of a substantial reduction in uptake appeared after 3 h; obvious drooping of leaves after 19 h; irreversible wilting and chlorosis on living leaves, after 43 h; and necrosis

Table 1 Correlation between production of cerato-ulmin and pathogenicity of *C. ulmi*

Strain	Spore production ml ⁻¹ ($\times 10^6$)	Index of cerato-ulmin production*	Indication of pathogenicity†
CESS 13K	1,267	265	+§
CESS 14K	1,266	118	+§
CESS 16K	1,426	278	+§
CESS CH5	1,536	118	+§
CESS PM1	1,323	140	+§
CESS T3	1,281	140	+§
CESS W1	1,283	300	+§
P1	949	1	±§
Q412	1,369	8	±§
W2 (Gloucestershire)‡	1,066	304	+
W4 (Essex)‡	1,047	337	+
W7 (Cambridge)‡	1,313	6	±
W9 (Oxfordshire)‡	1,367	6	±

Cultural conditions were the same as reported previously⁸ except that the level of the carbon source (sucrose) was shifted to 2%. Age of cultures was 1 week. Turbidity was measured at the wavelength of 400 nm using B & L Spectronic 20.

*Calculated as (turbidity) $\times$ (dilution factor of the sample for measurement) $\times 100$.

†Expressed as + (highly pathogenic) or $\pm$ (weakly pathogenic).

‡British strains.

§After Kondo (unpublished).

||After Gibbs and Brasier³.

on living leaves after 91 h. When the xylem was checked for brown discoloration at the end of the experiment (168 h after injections were begun), it was detected in all treated cuttings. No abnormal symptom was found in the control cuttings injected with distilled water however.

As cerato-ulmin seemed to be significant in disease development, the elucidation of the relationship between cerato-ulmin production and the pathogenicity of *C. ulmi* strains was of great interest. There seems to be no correlation between spore production and either cerato-ulmin production or pathogenicity (Table 1). Production of cerato-ulmin was found to be either high or low according to the method of measuring used. On this basis, *C. ulmi* strains were divided into two obviously different groups: high production and low production. A striking fact is that all strains with high cerato-ulmin production were in the highly pathogenic group, while all strains with low cerato-ulmin production were in the weakly pathogenic group. This relationship seemed clear-cut with no overlap between the two groups on the basis of cerato-ulmin production and pathogenicity. It should be noted that, of the four British strains, two are highly pathogenic (aggressive) and two are weakly pathogenic (non-aggressive)^{3,4}; these clearly separated in exactly the manner described above. Although there is variation in the production of cerato-ulmin in the high-production group of strains, it is not clear if intermediate production represents moderate pathogenicity of the strain because comparative data are insufficient for testing pathogenicity of those strains.

The ability of cerato-ulmin to produce symptoms of toxicity in white elm seedlings similar to those produced by Dutch elm disease suggests that cerato-ulmin could be responsible for disease symptom development in infected elm trees, if one assumes that the metabolite is produced *in vivo*. If this is the case, certain factors involved in cerato-ulmin production are inclined to induce symptom development. It is produced extracellularly and this ensures that there is good contact between cerato-ulmin and host tissues; because it is specific for *C. ulmi* it can direct specific symptom development, and its production during active fungal development is linked to its rapid production by the actively developing fungus within the host tissues. (Active development of the fungus is generally supposed to be dominant at the early stage of disease development.)

Together with the ability of cerato-ulmin to produce symptoms similar to those of Dutch elm disease, the correlation between cerato-ulmin production and pathogenicity indicates that differential production of cerato-ulmin is possibly responsible

for variations in pathogenicity among *C. ulmi* strains. On this basis, the production of cerato-ulmin might be a very useful criterion for fungal pathogenicity. Work is in progress to ascertain if it is applicable in a wider range of strains of *C. ulmi* than are presented here.

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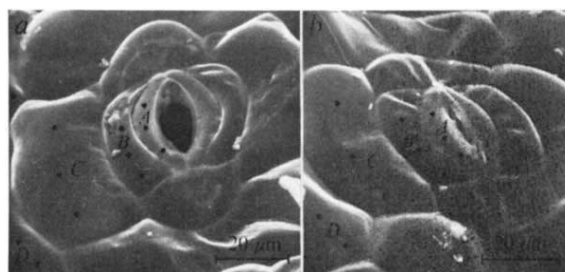


Fig. 1 Scanning electron micrographs of, *a*, an open and, *b*, a closed stoma of *C. communis* showing the approximate positions of point analyses in, *A*, a guard cell; *B*, an inner lateral subsidiary cell; *C*, an outer lateral subsidiary cell; and, *D*, epidermal cell.

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Stomatal movements and ion fluxes within epidermis of *Commelina communis* L.

STOMATAL movements are brought about by differential changes in turgor pressure between guard cells and other epidermal cells^{1,2}. The mechanism by which the turgor changes occur has been investigated for well over a century and a number of hypotheses have been proposed but none has been found to be completely satisfactory. Research in recent years has provided increasing evidence that active ion transport plays a major role in the stomatal mechanism³⁻⁶. It has been shown that, as stomata open, certain ions move into the guard cells and that this ion influx may be a dominant factor in raising the guard cell osmotic concentration. As a result, water enters the guard cells and brings about the opening movement. During closure of stomata the process seems to reverse whereby ions are lost by the guard cells. Cl^- and K^+ seem to be the major ions involved in the stomatal mechanism^{4,6-10}.

Humble and Raschke¹⁰ observed that in guard cells of *Vicia faba* the Cl equivalents were considerably less than were needed to balance the K equivalents; a similar situation existed in the guard cells of *Zea mays* although the K-Cl imbalance was not nearly so great¹¹.

Although *Commelina communis* has been used intensively for studies into stomatal mechanisms, assessment of the ion fluxes and charge balances involved have been neglected. Here we examine the fluxes of K, Cl, Ca and P between guard cells, subsidiary cells and epidermal cells of *Commelina communis* L. during stomatal movements. The results are discussed in relation to the K-Cl balance within the cell types in a closed and open stomatal apparatus.

Leaves from *C. communis* grown in the greenhouse in a peat-vermiculite mix supplemented with a complete fertiliser were used in the experiments. To obtain open stomata, the youngest, fully expanded leaves were floated on deionised water, abaxial surface downwards and illuminated from above for at least 3 h. Closed stomata were obtained by placing potted

plants in a darkened cupboard for 3 h. Before the epidermis was detached the leaves were examined under a microscope at low power ($\times 100$) to ensure that the stomata were open or closed. The abaxial epidermal strips, cuticle side uppermost, were placed on to carbon disks and rapidly frozen in liquid nitrogen. The epidermis, still attached to the carbon disks, was then freeze dried for not less than 60 min at -40°C under a vacuum of 0.05 mm mercury. After coating with carbon (approximately 100 nm thick) a dispersive-type analysis for the elements was made in the various cells of the epidermis (Fig. 1) using a scanning electron microscope with a solid-state X-ray detector attachment (Acton Ms-64 electron probe). The microscope was operated at 18 kV accelerating voltage with a sample current of approximately 100 nA. The take-off angle was 18° .

After obtaining secondary electron images of the stomata (Fig. 1), spot analyses (100 s counts with a spot size of 1 μm) were made at three positions (Fig. 1) in each of the following cells; guard cell, inner lateral subsidiary cell, outer lateral subsidiary cell and epidermal cell. This procedure was repeated three times for an open stoma (13 μm aperture) (Fig. 1a) and a closed or nearly closed stoma ($>1 \mu\text{m}$ aperture) (Fig. 1b). Potassium X-ray intensity slowly decreased with time due to volatilisation and so care was taken to initiate counting at the same relative time on each point. Thus, the error inherent in an analysis was comparable in each measurement.

Solid state X-ray spectrometers (used in this study) lack the energy resolution of the crystal spectrometers of the electron-microprobe analysers and although they measure through the whole spectrum simultaneously there is some interference between elements being measured. In efforts to minimise this the specimens were coated with carbon rather than metals which are normally used to obtain secondary electron images.

Both Ca and P are considered to be relatively immobile¹², a contention supported in these studies by the relatively constant

Table 1 Levels of K, Cl and Ca based against cell P levels and the K-Cl ratios in different cells of the epidermis of *C. communis* when the stomata were open (13 μm) and virtually closed ($>1 \mu\text{m}$)

Ratio	Guard cell	Inner lateral subsidiary cell	Outer lateral subsidiary cell	Epidermal cell
		Open stomata		
K-Cl	1.29 $\pm$ 0.16	1.65 $\pm$ 0.44	1.99 $\pm$ 0.72	2.20 $\pm$ 0.60
Ca-P	1.83 $\pm$ 0.36	1.92 $\pm$ 0.38	—	—
K-P	5.37 $\pm$ 0.83	2.37 $\pm$ 0.38	2.43 $\pm$ 0.71	3.04 $\pm$ 0.79
Cl-P	4.22 $\pm$ 0.85	1.47 $\pm$ 0.21	1.24 $\pm$ 0.10	1.42 $\pm$ 0.28
		Closed stomata		
K-Cl	1.88 $\pm$ 0.15	1.97 $\pm$ 0.33	2.47 $\pm$ 0.50	2.99 $\pm$ 0.44
Ca-P	1.86 $\pm$ 0.66	1.93 $\pm$ 0.59	—	—
K-P	3.21 $\pm$ 0.41	2.79 $\pm$ 0.43	3.75 $\pm$ 0.67	4.03 $\pm$ 0.56
Cl-P	1.70 $\pm$ 0.18	1.43 $\pm$ 0.15	1.57 $\pm$ 0.42	1.38 $\pm$ 0.27

Each figure represents the mean of nine determinations with its accompanying standard deviation.

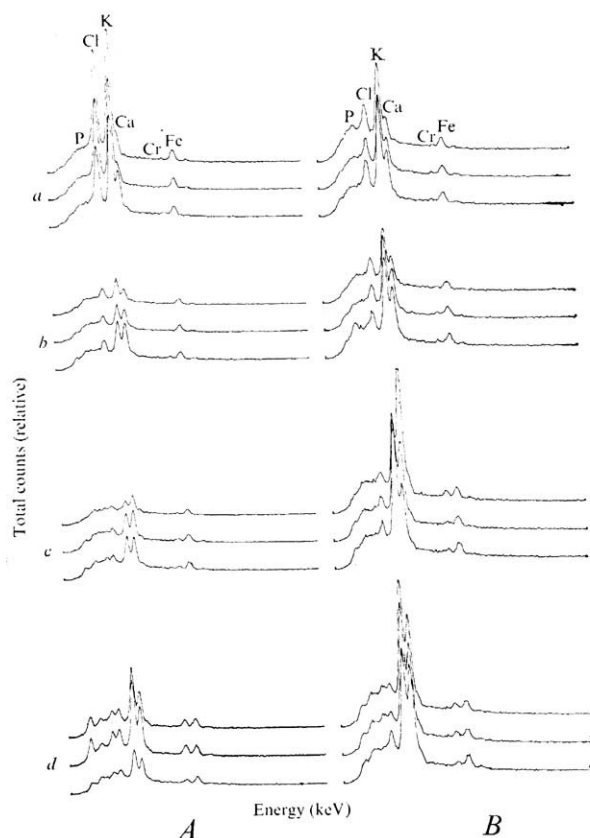


Fig. 2 Representative X-ray spectra of a microprobe analysis (three points per cell) of, *a*, a guard cell; *b*, an inner lateral subsidiary cell; *c*, an outer lateral subsidiary cell; and, *d*, an epidermal cell of *C. communis* with an open stoma, *A*, and a closed stoma, *B*. The Cr and Fe peaks are from the pole piece of the microscope.

Ca-P values observed in guard cells and inner lateral subsidiary cells when stomata were open and closed (Table 1). Ca-P ratios for the outer lateral subsidiary cells and epidermal cells could not be obtained because these cells contain large crystals of calcium oxalate and frequently the electron beam would hit a crystal and send the counts off scale (Fig. 2*c*, column *B*). Since the sample current varied slightly for specimens with open and closed stomata, peak heights for each of the elements could not be directly compared. Therefore, the K and Cl peaks were based against the P peaks which were considered constant from cell to cell. By expressing the K and Cl levels against P (which acts as a 'standard') errors which would be caused by the increase in guard cell volume when stomata are open are also largely eradicated.

Figure 2 shows representative X-ray spectra of the different cell types associated with an open stoma, *A*, and a closed stoma, *B*. A qualitative picture of the change in the levels of the different elements in each of the cell types associated with the open and closed stomata can be deduced from the peak heights.

Table 1 bases the peak heights of the elements against the P 'standard' enabling a more quantitative picture of the levels of the various elements within the cell types to be obtained. Highest levels of K were observed in the guard cell of the open stoma with least in the neighbouring inner and outer lateral subsidiary cells. In the case of the virtually closed stoma, K levels in the guard cell decreased relative to the open stoma while levels in the inner lateral subsidiary cell, and particularly the outer lateral subsidiary cell and epidermal cell, increased. Except for the guard cell of the open stoma which possessed the highest Cl levels, Cl levels were similar in all the cell types regardless of whether the stomata were open or closed. Thus, when a stoma of *C. communis* of aperture 13 μm closes to about 1 μm in response to darkness, K moves out of the guard

cell setting up a flux across to the epidermal cell. The reverse flux would occur upon opening. It would be presumed that Cl follows a route similar to that of K although, in these studies, Cl levels were not observed to increase in subsidiary and epidermal cells upon stomatal closure. Possibly Cl moves rapidly to other epidermal and/or mesophyll cells and does not accumulate in the subsidiary cells as K does. Only when ions reach epidermal cells are they able to enter mesophyll cells directly since the sub-stomatal cavity underlies guard and subsidiary cells.

Such a pathway for K (and possibly Cl) movement poses questions regarding the position(s) of the ion pump(s). A pump system presumably could be located in the guard cell plasmalemma which would maintain K gradients between it and the adjoining cells. Alternatively, a pump could be located in each cell type. A further consideration is whether the ions move between the cells through plasmodesmata and/or through the cell-free space.

Thus, the ion fluxes in *C. communis* do not seem to be similar to those in maize in which there is a movement of K and Cl between a pair of subsidiary cells and guard cells during stomatal movements¹¹. The grasses in general, in fact, seem to possess a K shuttle between guard cells and subsidiary cells during stomatal movements¹³.

K-Cl ratios in the different cell types increased in the order of guard cell < inner lateral subsidiary cell < outer lateral subsidiary cell < epidermal cell. This order was maintained regardless of whether the stoma was open or closed (Table 1). Thus, there was a greater imbalance of the two ions in epidermal cells. This imbalance of K-Cl also tended to be greatest in all the cells associated with the closed stoma. If organic acid anions bring about electrochemical neutrality balancing the excess K, as Humble and Raschke¹⁰ have suggested in epidermal cells, then it would be expected that the production of such anions must be even greater in epidermal cells. Evidence so far does not support this contention, however, since enzymes involved in the production of malate and certain other organic acids seem to predominate in the guard cells rather than the epidermal cells¹⁴. Presumably, however, organic acid fluxes from guard cells to the epidermal cells could overcome this problem.

We thank Drs J. Brown and J. Johnson of Georgia Technical University for their help in the operation of the microprobe.

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Carbohydrate-binding protein from *Polysphondylium pallidum* implicated in intercellular adhesion

INTERCELLULAR adhesion is generally believed to be mediated by specific macromolecular components on the surface of the interacting cells. Soluble factors which may play a part in this process have been identified in a number of systems¹⁻⁸. We have already described a developmentally-regulated⁹ carbohydrate binding protein^{10,11} from the cellular slime mould *Dictyostelium discoideum* that appears in differentiating amoebae in close correlation with the development of cohesiveness¹⁰. This protein is assayed by its erythrocyte agglutination activity that can be inhibited by specific sugars. We now report the isolation of a similar protein from another cellular slime mould, *Polysphondylium pallidum* that seems to mediate specific cellular adhesion as judged from the following findings: (1) it is present on the cell surface; (2) it is present when the cells are differentiated to a cohesive state but absent when the cells are not cohesive; (3) addition of the purified protein promotes cell cohesion; (4) sugars which react with the active site of the molecule block cell cohesion produced either by the added purified substance or by the endogenous substance present on cohesive cells; (5) the precise reactivities of the protein from *P. pallidum* differ from the protein from *D. discoideum*, which correlates with the segregation of these cells in mixed culture.

P. pallidum, like the other cellular slime moulds, exhibits two distinct phases in its life cycle¹²⁻¹⁴: a non-social vegetative phase and a cohesive phase, initiated by starvation. To generate vegetative and cohesive cells, 10⁶ spores of *P. pallidum* WS-320 in association with pregrown *Aerobacter aerogenes* were inoculated on to standard medium (SM) agar plates¹⁵ (100 mm diameter) and incubated in a moist atmosphere at 22° C in the dark. Cells in the growth phase (or not far from it) were collected from plates after 63 h of incubation when their density was fairly low (7 × 10⁷ per plate), washed in cold H₂O and separated from bacteria by centrifugation. The amoebae were differentiated¹⁶ into the aggregation-competent state by suspension (10⁷ ml⁻¹) and reciprocal shaking in 0.0167 M Na₂HPO₄-KH₂PO₄, pH 6.0. At intervals cells were removed for assay of cohesiveness and to be extracted for erythrocyte agglutination activity (Fig. 1). The cohesiveness assay, modified from one described previously¹⁰, measures the size of clumps formed from single cells passively brought into contact by roller-tube agitation. It has been demonstrated^{20,21} for *D. discoideum* that the size of free suspension agglutinates formed from single cells in the presence of EDTA correlates with the ability of the cells to form morphogenic contacts.

Table 1 Sugar effects on agglutination of erythrocytes by discoidin or *P. pallidum* agglutinin

Sugar	Sugar concentration for 50% inhibition of agglutination (mM)	
	<i>P. pallidum</i> agglutinin	<i>D. discoideum</i> agglutinin
Lactose	1.6	12.5
α Methyl-D-galactose	6.2	6.2
D-Galactose	6.2	25
N-acetyl-D-galactosamine	25	1.6
D-Fucose	25	3.1
L-Fucose	50	12.5
3-O-methyl-D-glucose	100	1.6
α Methyl-D-glucose	≥ 100	12.5
β Methyl-D-glucose	≥ 100	100
D-Glucose	≥ 100	≥ 100
D-Glucosamine	≥ 100	≥ 100
N-acetyl-D-glucosamine	≥ 100	≥ 100
D-Mannose	≥ 100	≥ 100
α Methyl-D-mannose	≥ 100	≥ 100

Purified *P. pallidum* agglutinin and discoidin¹¹ were assayed for agglutination activity as described in Fig. 1 in the presence of a series of concentrations of sugars to determine the sugar concentration required to inhibit agglutination activity by 50%.

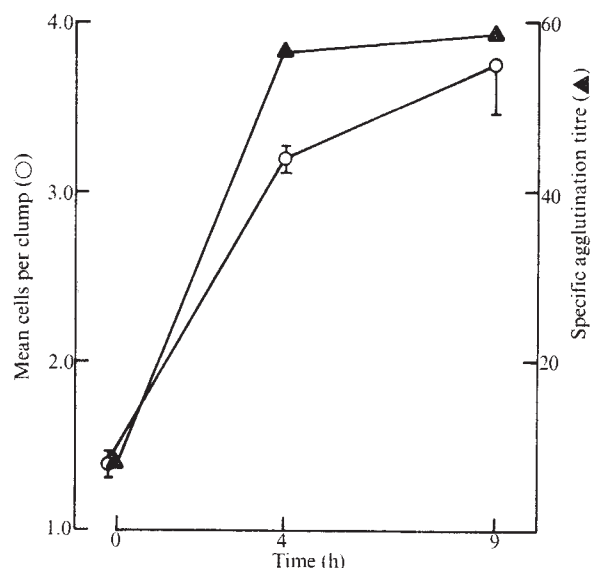


Fig. 1 Agglutinin activity (▲) and cohesiveness (○) during development of *P. pallidum* in suspension culture. Cells maintained on SM plates¹⁵ with bacteria for 63 h were washed free of bacteria and inoculated in suspension medium without bacteria¹⁶. Aliquots of differentiating cells were removed for assay at the times indicated. For the agglutination activity assay extracts were prepared by sonicating 3 × 10⁸ cells (Bronwill Sonifier with needle probe; four bursts of 25 s each at intensity 50) in 3 ml of 75 mM NaCl, 75 mM KCl, 1 mM EDTA, 15 mM Tris-HCl (pH 7.3)¹⁷ containing 0.3 M D-galactose. Extracts were centrifuged at 150,000g for 45 min (4° C). Supernatants, after extensive dialysis against 75 mM NaCl, 75 mM Na₂HPO₄-KH₂PO₄ (pH 7.2) (PBS) were assayed with formalinised human red blood cells¹⁸ (FHRBC). Assays were performed in Microtiter V plates (Cooke Engineering) using serial twofold dilutions of the extract. Each well contained 25 µl of saline (0.15 M NaCl) plus 25 µl of extract diluted in PBS to which 25 µl of a 2.5% (v/v) suspension of FHRBC in PBS was added. The patterns were read after 1.5 h. Titre was expressed as the reciprocal of the highest dilution giving positive agglutination. Specific agglutination activity was expressed as titre divided by protein concentration. Protein concentration was determined by the method of Lowry *et al.*¹⁹ with bovine serum albumin as a standard. For the cohesiveness assay the procedure was as follows: (1) Cells collected from the suspension culture were washed in water (4° C) and suspended in EDTA-phosphate buffer (16.7 mM Na₂HPO₄-KH₂PO₄, 10 mM EDTA (pH 6.2)) as modified from Gerisch²⁰. (2) The suspension was dispersed into single cells by repeated pipetting through a fine-tipped pipette and was adjusted to a concentration of 5 × 10⁶ cells ml⁻¹. 1 ml of this suspension was diluted 50-fold in EDTA-phosphate buffer and counted with Coulter counter (Model ZB-1) using a 200 µm aperture with 1/aperture current at 1/16, 1/amplitude at 1/8 and the lower and upper thresholds set at 20 and ∞. (3) 5 ml of the cell suspension was added to a 17 × 150 mm glass test tube. The tube was rolled about its long axis at 20 r.p.m. for 30 min (23° C). (4) The contents of the tube were diluted 20-fold in EDTA-phosphate buffer and counted in the Coulter counter at the above settings. With coincidence corrections, the ratio of the total number of particles before and after rolling was computed. This ratio is the mean number of cells per clump and is taken as an index of cell cohesiveness. The standard error of the mean for three separate determinations is shown.

Cohesiveness of cells and specific agglutination activity in cell extracts increased in parallel after separation from bacteria (Fig. 1). In subsequent experiments, we found that synchronous growth-phase cells grown in suspension with bacteria¹⁶ under conditions in which all cells had equal and ample bacterial food, contained no detectable agglutination activity. Thus agglutination activity was absent in vegetative cells, but appeared in food-deprived, differentiating cells.

To purify agglutinin we cultured cells on SM plates for 90 h in the dark. Under these conditions the cells become cohesive but do not develop beyond broad, loose aggregates. We sonicated the cells in 0.15 M NaCl, 0.3 M galactose, adsorbed the dialysed supernatant with formalinised erythrocytes and

(Continued on page 149)



Book Review Supplement

THE prefix 'para—', says the OED, means "beside, beyond, wrong, irregular". Excellent—just right for the terms 'parascientist' and 'parathinker'.

Tact prevents my naming present-day parascientists and parathinkers: but anyone can see that some of them at least must be identified by the field of intellectual endeavour in which they operate, that is to say in interpreting what I shall call the 'it-makes-you-think-don't-it?' type of seemingly natural occurrence such as being given one's grandmother's maiden name by a clairvoyant, seeing Uri Geller bend teaspoons, or guessing (beyond statistical prediction) what the next card but one is going to be that somebody turns up in the next room. If the last of the three actually does happen to hold water from a statistical point of view, it remains egregiously boring and useless: it doesn't lead to anything. And the first two are drawn from a field of human activity that is, alas, far too wide-open to illusion, charlatanry and fraud.

But the serious point I want to make is something other, something which underlines my scorn for parascientists and parathinkers. It's this. So much of the interpreting, let alone the theorising, of parascientists and parathinkers is based on seemingly natural occurrences which either cannot be fully corroborated according to the rules of evidence, or cannot be properly repeated according to the rules of experiment—or, of course, both. Yet this is not to say that to me the most striking thing about parascientists and parathinkers is their being beside, beyond, and so on; lots of scientists and thinkers have been all of those. (Think of Newton on numerology.) What to me seems the most striking thing about them is what makes them lurable into the beyond, beside, irregular and wrong: namely an astonishing credulity. The critical link between 'it-makes-you-think' and 'there-must-be-something-in-

All parasense

William Cooper

Aldous Huxley: A Biography Vol. 2: 1939–1963. By Sybille Bedford. Pp. xii+378+15 photographs. (Chatto and Windus, in association with Collins: London, 1974.) £4.50.



Aldous Huxley. A portrait by Alfred Wolmark, drawn in 1928. (National Portrait Gallery)

it' seems to me indefensibly weak, if not totally absent. I don't understand it: they include some very clever men. Here speaks one of them:

"Elinor Bond doing telepathic guessing . . . Frances Farrelly, with her diagnostic machine . . . Harry, the Dutch sculptor, who goes into trances in the Faraday cages and produces automatic scripts in Egyptian hieroglyphics; Narodny, the cockroach man, preparing experiments to test the effects of human telepathy on insects . . . It was all very lively and amusing and, I really think, promising . . ."

All very lively and amusing is for him to say, naturally. Promising—we can have an opinion on that. The only

think it surely promises is more of the same nonsense. The speaker is Aldous Huxley.

Now we all have our off-moments, and the quotation, which comes from the second volume of Sybille Bedford's biography of Aldous, may not be entirely fair. But it is not entirely unfair. The part played in the second half of Aldous's life by—shall we call it?—parasense, in whatever degree of light-heartedness or seriousness, is too great to allow that he be absolved from the charge of astonishing credulity. It is clear that he, being one of the most intelligent of men, was singularly free from pomposity, ill-will and aggressiveness: all his friends loved him as selflessly thoughtful and harmonious almost to the point of saintliness. The condition of men in the twentieth century, Isaiah Berlin observes, was the single theme that dominated his late years. And beyond warnings to mankind—first of nuclear holocaust and then of ecological catastrophe—Aldous's message for each of us, personally, delivered with a most taking humility and amused down-to-earthness was: Try and be a little kinder to each other. Hard to resist, I must say. True, there was something remote about him: as everybody has pointed out, he scarcely noticed that his first wife, Maria, was dying of cancer. (On the other hand he too died of cancer with great dignity.) Yet . . . why did he allow himself to be so credulous about paranormal occurrences, about 'mind-changing' drugs and the like? The only person to give any sort of answer is Charles Snow, who suggests "just one touch of immodesty. . . . If he kept his mind open beyond the limits of openness, could he perhaps happen on something earth-shaking, altogether outside the common run of life? And enjoy announcing it to an astonished world? Like a more dramatic T. H. Huxley about something more exciting than Darwin's

Theory?" It's hard to come up with a more likely answer. Poor Aldous: it never came off; it never could.

So Mrs Bedford arouses our compassion. Over my regard for her as a biographer I shot my bolt when reviewing Volume 1 in this journal. I see myself quoted on the dust-jacket of Volume 2: "Mrs Bedford has great literary talent and through everythink she writes shines a sympathetic intelligence—not to say an admirably unforced professionalism as well". I stick to that. Over a final verdict on the biography I naturally insisted on waiting until the end. The doubts I had at that time have not really been resolved now that I have read the second volume. I noticed that the book has the flavour of close, cosy, penetrating gossip: all well and good—but I feel that in the end it becomes too much of a good thing. There are too many gossippy letters from Maria, too many long quotations from other people's letters. And for my taste there is too much detail—Joe Blodgett-ish, American academic, research detail—about Aldous's incessant, restless lecturing tours. (Did he really have to travel so much, and to live in southern California, in order to make a living and maintain his health? Such a life was particularly bad for him; he ought to have stayed put, among more stable-minded men of his own intellectual stature, in a less fatuous culture.)

Finally, I get a recurrent feeling that Mrs Bedford, who knew Aldous well and was a close friend, is somehow in this volume shielding him, protecting him from criticism. It's wholly understandable, and it's permissible for part of the time; but there's another part of the time when a biographer must come clean, and cold—or so it seems to me. All the same, Mrs Bedford has written a remarkable and valuable book.

Science from the beginning

Early Physics and Astronomy: A Historical Introduction. (History of Science Library.) By O. Pedersen and M. Pihl. Pp.413. (Macdonald: London; American Elsevier: New York, August 1974.) £10.95.

THE content of this revised and updated English version of an originally Danish text is summarised well in the description on the dust jacket of the book. "The first section of the book is devoted mainly to Greek physical science . . . Concepts of nature and of scientific laws in general are discussed in addition to theories within particular sciences. The reader is also introduced to the mathe-

matical and other techniques available for the solution of problems. The second section of the book discusses the transmission, reception and elaboration of these theories in the Latin West, and the science of the early Renaissance is seen as the end-point of the classical tradition. The book concludes with biographical sketches of the philosophers and scientists featured in the text."

Not everyone would agree with the authors' prefatory remark that their omission of a detailed account of early Egyptian and Babylonian science was "inevitable", though it was doubtless expedient for them to limit their already extensive theme. Probably for the same reason they omitted any mention of the rich scientific developments in mediaeval India or China, the early history of pure mathematics, time-reckoning, cartography, and so on. It is, on the other hand, truly inevitable, and a little unfortunate, that Alexander Thom's important researches on megalithic lunar observatories and solstitial sites have been published too recently for inclusion in the text or in the bibliography (updated to 1970).

In the treatment of Greek science emphasis is laid upon the importance of the presocratic belief that causal relationships existed between natural phenomena. Aristotle's adherence to this causal principle (or natural law), coupled with the trend away from mythology towards rational explanation and the Platonic concept of the mathematisation of nature, constituted essential ingredients of subsequent scientific thought. Menaechmos's discovery of the conic sections (in about 350 bc) may have arisen from the study of the daily shadows cast by a gnomon upon the ground. That was a hidden empiricism which is also detectable in the Pythagorean relationships between number and harmony, the Method of Archimedes, and the pneumatical experiments of Strato Philo and Hero at Alexandria during the Hellenistic period of Greek culture.

It was, however, mediaeval investigations in optics and statics, founded upon principles drawn from everyday experience, which provided the firmer empirical basis of physical science. The plane astrolabe and mechanical clock were but two of many mediaeval inventions destined to revolutionise astronomical practice; and the rejection of the Aristotelian belief in a causal principle for the explanation of motion was the determinative factor in the development of dynamics, which was required to make the physical implications of Copernican astronomy acceptable to Galileo and many of his contemporaries.

The field which is explored is **much** more complex and extensive than this outline may suggest, and is already familiar to students of early science. The well balanced presentation in one volume is a welcome substitute for the wide range of scattered publications from which the subject matter would otherwise have to be drawn. The extensive bibliographical and biographical references provide a very useful guide to carefully selected primary and secondary sources. The use of vector notation to simplify explanations of the various geometrical devices used by Greek and mediaeval scholars, though a useful heuristic tool, is anachronistic and presupposes that the reader has received instruction in this technique.

I detected only six minor printing errors in the text, but slightly less care seems to have been taken by the authors and publishers in connection with the description of a great many of the accompanying figures.

The authors' hope that this book will become widely used as a standard text may be dashed because of its price. The onus is likely to fall upon teachers to obtain library copies and to arrange that these are easily accessible to the members of their classes. My own experience leads me to believe that the clear and scholarly style of this excellent translation, along with the authors' practice of breaking up the text into numerous sub-sections, will appeal greatly to science undergraduates reading the book as part of an introductory survey course in the history of the exact sciences.

Eric G. Forbes

No matter—never mind

The Brain Revolution. By Marilyn Ferguson. Pp. 380. (David-Poynter: London, 1973.) £4.00.

THIS book reviews the flotsam and jetsam discovered by those working on the remote shores of the brain sciences. Among other things, it tells us that "human volunteers in biofeedback laboratories are learning consciously to control their brain waves, perspiration, blood pressure, digestive juices and heart rate" (p.17): anyone intending to read it would be well advised to acquire such control first. The authoress tells us that "breakthrough scientists ask silly questions" (p.286) and the book parades before us their silly answers. The human psyche is said to be influenced by star patterns, magnetic fields and negative ionisation; precognition and telekinesis are accepted scientific phenomena; the "incredible potency of LSD" (p.123) cures

psychoses, autism and alcoholism (though according to one study the last cure only occurs "in 50% of cases" since only one out of the two alcoholics treated actually improved, p.124); we can perceive colour with our finger tips when we are not using them for curing cancer by electrical emanations; and we can of course learn effortlessly either by eating "respected scholars" or by having linguaphone records played to us whilst we are asleep.

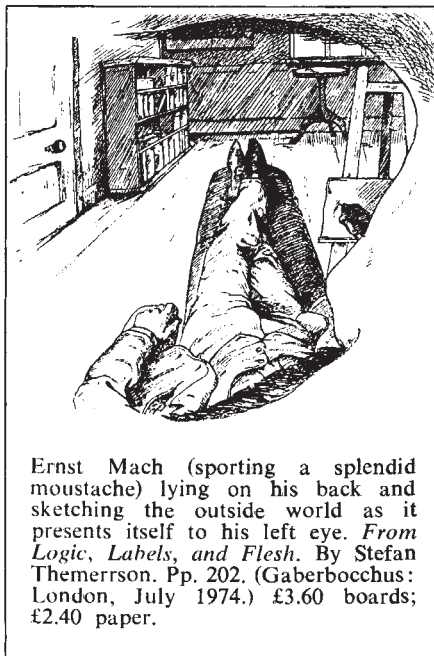
The language in which the book is written suggests that the authoress may herself have been in ASC (altered state of consciousness) throughout its preparation. Scientists are alternatively "stunned" and "baffled"; discoveries are "astounding", "amazing", "incredible" or "one of the biggest breakthroughs of our times". Her style speaks for itself: "Into the whirlpool of eddying, oscillating, hormones of a human female toss a contraceptive pill. The vigilant brain, taking astonished note of the suddenly escalated progesterone and oestrogen, behaves as if pregnancy has occurred" (p. 219).

Even when she deals with well established phenomena, such as size constancy, the limits of the visible spectrum, or the after-effects of seen movement, Marilyn Ferguson can do no more than invite us to gawp at them: no attempt is made to provide the reader with an understanding or an explanation, and mysteries are conjured up where none exist. In spite of reservations about the way she presents her material, one cannot but admire her industry: there is a 23 page bibliography in which the citations range from *Science*, through *Esquire* to the *Psychedelic Review*.

Although this book cannot be taken seriously, it may be worth asking whether it matters that so much effort is devoted to making specious discoveries and publicising nonsensical theories: after all, the brain may work in ways that are at variance with existing scientific paradigms, and unless someone produces and tests unlikely hypotheses, we would never know. It may even be that some of the unusual findings reported in this book will eventually be validated. Moreover, in the long run science will catch up and false hypotheses and observations will be discarded. In the meantime, it is only the gullible, those in search of a mystery or a sensation, who will give credence to this kind of work: the rest of us either suspend judgement or hear on the grapevine about all the negative results that do not get into the journals.

Sensation mongering research often does, however, have bad effects. First, much of it, particularly in Russia and the United States is funded from public

money which might be better spent elsewhere. Second, scientists may feel a duty to investigate claims made by others, and if the original research is bad this may waste a great deal of time and effort: proving that an effect does not exist is notoriously difficult. It is said that a Nobel prize winner spent three years attempting to replicate some of the experiments on cannibalism in *planaria* without success. Third, current trends to mysticism are given a scientific prop. Not all mysticism is bad but mysticism based on bad science is likely to be bad mysticism. Fourth, and most important, when the 'discoveries' are in the brain sciences, they often fall into the hands of medical practitioners, the most gullible of professional people; and when applied to human patients such discoveries can cause a great deal of suffering. Leeches and calomel have now



Ernst Mach (sporting a splendid moustache) lying on his back and sketching the outside world as it presents itself to his left eye. From *Logic, Labels, and Flesh*. By Stefan Themerson. Pp. 202. (Gaberboechus: London, July 1974.) £3.60 boards; £2.40 paper.

been abandoned, prefrontal leucotomies and insulin-induced coma are on the decline, but psychoanalytic therapy still thrives in the face of the evidence, and there is an increasing fashion for amygdaloid lesions and for maintaining children with that mysterious syndrome "minimal brain damage" on large doses of amphetamine.

Perhaps the research councils should establish teams of sceptical but tolerant scientists prepared to move to trouble spots and investigate, in collaboration with the original discoverers, any seemingly outrageous claims that might be of importance either for science or human life. No doubt, on most occasions, they would merely put out the flames expeditiously, but occasionally a really important discovery might be rescued from scepticism, and

at least we might have firm answers to such questions as whether acupuncture works by some process other than blind faith, or whether learning can be transferred from one animal to another by injecting a homogenised brain extract. It does seem extraordinary that no definitive answer to this last question can be given 15 years after it was first raised.

N. S. Sutherland

Neuroteleology

Logic of the Living Brain. By Gerd Sommerhoff. Pp. ix+413. (Wiley: London and New York, 1974.) £5.75.

IN 1950 Sommerhoff published a book entitled *Analytical Biology*: it deserves to be more widely read than it has been since it is one of the best philosophical analyses of teleological terms. He argued that a system may be said to be goal directed when its outputs correlate with variations in the states of the environment in such a way that for a wide range of environmental states the same end condition is achieved. He named this concept 'directive correlation' and tried to formalise the idea. His new book attempts a grandiose synthesis of brain and behaviour, and had it too been published 24 years ago, it would doubtless have met with the measure of critical acclaim that in that distant age greeted other *pot-pourris* of the kind, such as Hebb's *Organisation of Behaviour*. Like such books, it would also have been forgotten by now.

In the first section of his new book, Sommerhoff argues that biologists and psychologists have been unable to grapple with the real complexity of the brain because they have not had a set of objective scientific concepts appropriate for the description of a system that is goal directed and that forms and uses models of the outside world. He alleges that brain scientists, by avoiding all teleological language, either oversimplify the problem of how the brain controls behaviour or else they use teleological language in a confused and non-objective way and therefore produce muddled theories. Unfortunately, no instances are given of the muddles that arise and one can only speculate about who all these confused theorists are.

Sommerhoff's solution to this problem is two-fold: first, to make use of his earlier idea of directive correlation; and second, to construct nerve nets with classificatory functions that can change through learning. In the second half of the book, he looks at various behavioural phenomena and tries to show how they can be explained in terms of these two ideas.

The book has several serious faults.

Scientific Research in British Universities and Colleges 1973-4

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First, it is often wildly inaccurate. For example, it is stated that "The assumption that reinforcement can directly strengthen responses which precede it by a time interval of more than a few seconds is contrary to the evidence" (p. 167). Second, Sommerhoff seems for the most part to be unaware of recent psychological work and theorising. This vitiates his account of language and animal behaviour, whilst enabling him to set up as Aunt Sallies Hull and Tolman who, except from the view point of the historian, no longer merit serious discussion. Third, the book contains a number of errors in logic. For example, Sommerhoff argues that pattern recognition is based on eye movements: he claims (p. 272) that it is easier to translate the extent and direction of an eye movement into a directional vector than to translate a retinal distance: he forgets that, since saccades are ballistic movements, the brain must compute visual angle and direction from the retinal image before initiating the eye movement.

Finally, and most importantly, despite the block diagrams and mathematical flourishes, the concepts Sommerhoff himself introduces do not seem to provide any new explanations of known phenomena and are less powerful than the concepts and explanatory models in current use by workers in the field. Thus, his classificatory and learning networks are not powerful enough to cope with what is known about the visual perception of objects, and his introduction of the notion of 'expectations' in this context remains vague. On the other hand, he is clearly aware that perception does involve the problem of inference and the construction and accessing of representational models, but he does not refer the reader to any of the extensive work that has been undertaken on such problems over the last decade.

The basic neural networks suggested by Sommerhoff are simple, but they do have a certain elegance. For example, he shows how, with the use of recurrent or forward inhibition, it is possible to construct networks that have the properties of flip-flops. The difficulties begin when he tries to explain complex behaviour or information processing by making more elaborate models in which his simple networks are put together into complex structures. At this point, the models become far from clear, and it is impossible to know whether they would behave in the way that is claimed or not: the only way to find out would be to simulate the models on a computer but Sommerhoff has not attempted to do this. Indeed, he dismisses the analogy between brains and computers in a few pages early in the book. He points out several differences between the hardware of existing

computers and that of the brain, but this is not really what is at issue, since computers are so constructed that they can be programmed to model any logical system within the limits imposed by their storage capacity and processing time. Having in this way foresworn computer simulation, he leaves himself without any good tool for constructing and testing models of the processes that underlie behaviour.

Throughout the second part of the book, Sommerhoff makes a valiant attempt to tie up function with brain anatomy. He assigns more or less plausible roles to some of the known connections in the brain, but in terms of the complexity of the tissue and of the behaviour which is controlled the assigned functions tend to be rather simple. The relationship between the evidence and the theorising is, as he himself admits, frequently tenuous.

The book is well written, and should bring a glow of nostalgia to those who pine for the slap-happy fifties when we could raise two cheers for anyone bold enough to tell us how behaviour could be taken care of by some neural nets and a few connecting tracts.

N. S. Sutherland

Scientific rambles

Science and Values: Patterns of Tradition and Change. Edited by A. Thackeray and E. Mendelsohn. Pp. viii+251. (Humanities Press: New York, 1974.) \$11.00.

IN spite of its rather grand title, this collection of essays is a useful example of some pioneering work in the history of science. A more accurate title would have been "Science (or better, Scientists) in Social Context"; or even, considering parts of the text "What Some People Can Get Up To With Science". The editors disclaim any intention to air comprehensive or synthetic views; what they present are papers exploring strongly "contextual" approaches to the history of science from Rome to Tokyo, and from Manchester to Madagascar. This miscellany is the product of the Van Leer Jerusalem Foundation Conference in 1970.

With such rapid enlargement of the horizons of historical research it becomes difficult to find a theme common to even a few of the essays. The volume starts with a brisk general survey of the changes in English science since the eighteenth century leisured gentleman gave way to the nineteenth century worried middle class. There is a blow by blow description of the "Ayrton incident" of 1872, when the absolute supremacy of the Hooker dynasty at Kew Gardens was challenged by the eccentric Ayrton,

then Commissioner of Works. Thanks to Ayerton's removal because of an unrelated embarrassment, the scientific establishment escaped the humiliation of being rendered publicly accountable for their stewardship of public funds.

The most sensitive piece of 'externalist' historical writing comes from the American Charles Rosenberg, who describes the strategies of agricultural researchers returning from Germany in the 1850s. Here, we find cultural context, administrative constraints, and personal self-estimation, woven together very beautifully.

But the reader is not permitted to tarry on these familiar scenes; the anthropology of science beckons. Early nineteenth century Madagascar, its rulers anxious for 'magicians' to provide the tools of power, attracted a mixture of industrial, political and clerical entrepreneurs. This early "Technical Assistance Programme" failed to Europeanise the culture, though in a half century it did cause its decay.

Politics and changes in the weather

Weather Modification in the Public Interest. By R. G. Fleagle, J. A. Crutchfield, R. Johnson and M. F. Abdo. Pp. ix+88. (University of Washington: Seattle and London, July 1974.) £2.85; \$5.95.

Any good work of science fiction depends on one or two critical, and possibly fantastic, assumptions from which all the rest follows logically. In a similar way this study by Fleagle and his co-authors depends on the assumption that weather modification will become possible on a substantial scale, with reasonably predictable outcomes. Given this, the book discusses logically the political, legal, administrative and management structure which will be needed to control weather modification.

Some weather modification has already been demonstrated as practical—the clearing of cold fogs, for example—but that has raised few administrative problems. The seeding of orographic clouds to increase the snow and rain over mountains has also been practised for many years, apparently with some success, but that has not raised serious legal problems. If, however, a government agency could modify or divert hurricanes, or if it initiated cloud seeding on a grand scale then no doubt greater problems would arise.

There is a danger that the reader of this book who lacks a strong meteorological

background will be led to believe that the potential of weather modification is much greater than has so far been proved, which may lead him, or her, to accept as necessary the somewhat elaborate organisation recommended in the book.

In the few legal actions brought in the United States under the quite extensive legal framework already established, none of the plaintiffs has been able to establish a cause-effect relationship between the actions of the weather modifier and the alleged results. This points to the premature nature of any elaborate legal or administrative framework.

On the other hand, the well-briefed meteorologist will find much of interest in the history of weather modification in the United States and in the views of the authors on future developments. Whether or not one accepts the view that weather modification will be used on a wide scale in the future, an activity which currently involves the expenditure of around \$20 million of public funds in the United States certainly deserves public discussion. Furthermore, it is well to recall also that almost \$3 million is spent annually on cloud seeding by American companies operating abroad. The international aspects cannot be ignored: the effects may be greater on international relations than on climate.

J. R. Ravetz

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Post script

The Freud/Jung Letters. Edited by William McGuire. Pp. xlii+650. (Hogarth Press, and Routledge and Kegan Paul: London, 1974.) £7.95.

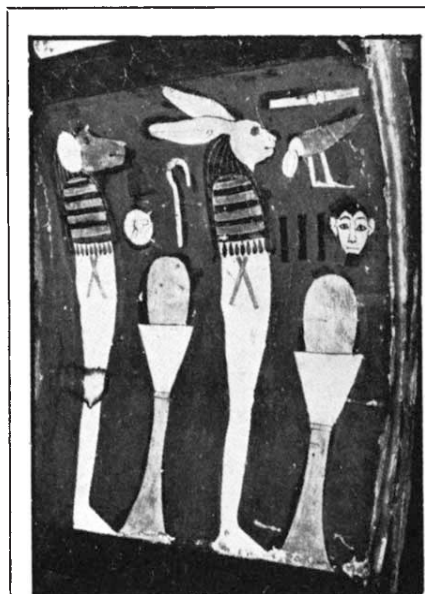
THE publication of this volume marks an important contribution to the history of the psychoanalytical movement, comparable to the issuing, in 1954, of Freud's letters to Wilhelm Fliess. Following his communications with Fliess, this exchange of material with his later protagonist, Jung, formed another chapter in the development of Freud's ideas. It is, perhaps, as the chronicle of a fruitful but doomed relationship that the volume has its greatest appeal, and as such, it forms a useful pendant to the official versions which have been given by Ernest Jones and by Jung himself.

When reading the letters, one is struck by the extent to which differences were managed for such a long time. For those familiar with the account of the break as given in *Memories, Dreams, and Reflections*, the tolerance demonstrated by Freud in the last years is unexpected. Yet the need felt by both men explains the concessions, the reconciliations, even the tensions that led to the final parting. A letter sent by Freud in 1907 sums up the situation beautifully:

"... now of all times I wish I were with you, taking pleasure in no longer being alone and, if you are in need of encouragement, telling you about my years of honourable but painful solitude... about the indifference and incomprehension of my closest friends, about the terrifying moments when I myself thought I had gone astray... about my slowly growing conviction, which fastened itself to the interpretation of dreams as to a rock in a stormy sea, and about the serene certainty which finally took possession of me and bade me wait until a voice from the unknown multitude should answer mine. That voice was yours" (p. 82).

Amid the intellectual torpor of the Burghölzi Hospital, Jung responded in kind. The meeting of two such gifted minds inevitably acquired complications, for as Jung looked to Freud in terms of a father figure, so too the older man regarded his younger colleague as a son and heir. Freud wanted Jung to explore schizophrenia in the way that he himself had studied obsessional neurosis (p. 168). But even though Jung had begun to do that before the correspondence began, his reservations about an exclusively sexual origin for mental illnesses were evident from the first (pp. 4-5, 7, 79, and 138-139). Later, as Jung developed his own theories about the mind, disagreements became more marked. After Jung's lectures in America in 1912, an open break was inevitable and it came within a year.

Publications and personalities fill



Hare-headed divinity of ancient Egypt on the coffin of Bakenmut, 21st dynasty. From *The Leaping Hare*. By G. E. Evans and D. Thomson. Pp. 262+24 photographs. (Faber: London, October 1974.) Paper, £1.50.

many of the letters, but the editor William McGuire aids the reader with footnotes which are concise as well as informative. It is a well produced book with lucid translations by Ralph Manheim and R. F. C. Hull, useful appendices, and an index. Finally, thanks should be given to the estates of both men for allowing the publication earlier than had been expected.

B. A. Boucher

Fifty year's minutiae

Half a Century of Medical Research. Vol. 1: Origins and Policy of the Medical Research Council (UK). A. Landsborough Thomson. Pp. xiv+308. (HMSO: London, June 1974.) £4.60.

WORTHY, rather than exciting, is the appropriate adjective for this book. It is the first of two volumes which will deal with fifty years of medical research in England. This first volume covers the history of the Medical Research Council and articulates the earlier principles of policy insofar as the future administration of the council and the future developments in scientific programmes are concerned. What comes out of this book—and, one can confidently expect, from the volume to follow—is almost every kind of fact and statistic that anyone interested in such matters could possibly hope for. Do you wish to know the details of the relevant Ministry of Health Act of 1919? Then this book will tell you just how this act was passed and how a very necessary legislative pro-

vision was inserted to cover the question of "The organization of enquiry and research on health activities". Do you wish to know the minutiae of the administrative organisation of the Medical Research Council: which sub-committee was formed to do this or that; that the title Second Secretary, first used in 1949-57 and revived in 1965, is now established for an officer able to act as the Secretary's second-in-command and deputy; that in 1914 rooms for temporary offices were taken at St. Stephen's House, Westminster, at a rent of £25.00 per annum, and that the council moved to Park Crescent in September 1961, into a building with a frontage of 255 feet with a depth of just over 42 feet? The book abounds in items like these.

As a living history I have to confess I find it lamentable, though as a compendium I find it invaluable. Nothing of the vibrating personalities emerges at all, nothing of people's real feelings, nor any real analysis of the infinite complexity of issues. So much is written up as a scientific *Jennifer's Diary*. We are told, for example, that on the occasion of the council's Jubilee Dinner, celebrated in November 1963, the Duke of Edinburgh was prevented from attending but that Lord Shawcross presided. In the absence of the Prince, The Right Honorable Quintin Hogg proposed the toast and a reply was made by Sir Henry Dale, but nothing of what was actually said on this occasion is recorded. Perhaps no-one really said anything—perhaps no-one knows; but I can hardly believe that. To add that: "The presence of Lady Fletcher and Lady Mellanby at the high table was particularly appropriate", may add something to our edification but nothing to our education.

This book is terribly safe; it says nothing controversial. There is very little that one can object to except that it is dull. Only a few notes of human vitality and spark emerge from the thumping melodic line. So, like drops of water in a desert, we read that the secretary of the council on February 2, 1939 wrote: "The discussion then wandered from the point and as we were both tired of cursing each other, we satisfied our minds by cursing everybody else". It is good to know that someone has a sense of humour. This is a book for the library but, unless you suffer from insomnia, it is not for the bedside.

I think it is a great pity that the sponsors have not been better served. The Medical Research Council, in achievements and policy, is an institution that we can contemplate with warmth and pride, and its history deserves a more vigorous and dynamic treatment.

June Goodfield

Astronomy for astrophysicists...

Gamma-Ray Astrophysics. Edited by Floyd W. Stecker and Jacob I. Trombka. (A symposium held at NASA Goddard Space Flight Center, April, May 1973, sponsored by NASA and the American Physical Society.) Pp. xvi+412. (Scientific and Technical Information Office, NASA, Washington, DC, 1973.) \$2.90.

ASTRONOMY has been one of the most prolific of modern sciences, but the gestation period of one of its offspring— γ -ray astronomy—has been painfully long. The conception was in 1958 but it was not until 1972, after many false alarms, that birth finally occurred. This book is the report of a symposium on γ -ray astronomy which was held at the Goddard Space Flight Center in April 1973, and, therefore, marks a significant stage in the subject.

The papers read at the symposium illustrate very clearly the current situation in γ -ray astronomy and the great differences between that subject and the neighbouring X-ray astronomy. X-ray astronomy began in 1962 with the unexpected discovery of an X-ray source outside the Solar System. This discovery was made with a detector of modest size on a short rocket flight; experimentalists immediately realised that, with the use of larger detectors and the much longer exposure times provided by satellites, a rich field was waiting to be exploited. By contrast, few results were forthcoming in γ -ray astronomy until the experimental techniques had been stretched almost to their limit. This aspect of the subject is illustrated by the experimental contributions to the symposium, many of which contain a great deal of discussion of the statistical significance of observations, and of the reality, or otherwise, of differences between experimental results.

Conference reports inevitably reflect the vices and virtues of conferences and this one is no exception. One of the worst aspects of conferences is the superficial manner in which each topic has to be treated because of the limitations of time; nearly all of the contributions to this symposium, both experimental and observational, have been treated much more fully in various journals. Many scientists believe that the real value of conferences are the discussions which take place outside the formal sessions. Obviously, this activity is not recorded in a report but something is retained in the reporting of the question time at the end of each session. Here, one finds a genuine element of criticism and dis-

cussion by Schwartz and Gursky of the mally in journals. A very valuable paper at the symposium was the discussion by Swartz and Gursky of the experimental problems encountered in measurements of the spectrum of the diffuse flux of cosmic γ rays. This feature was discovered as early as 1964 but the problems of eliminating the effects of atmospheric background radiation and the effects of cosmic ray interactions in the detector still have not been solved. A critical discussion of the experiments in this field was long overdue.

One of the breakthroughs in 1972 was the discovery by Chupp and his coworkers of γ -ray spectral lines from a flare on the Sun. A paper by Ramaty, interpreting these results, illustrates the potential value of measurements of this type to our understanding of the role of high energy particles in flares. There is a contrast between this theoretical contribution and others which discuss subjects such as the decay of radioactive nuclei in supernova remnants, the interactions of cosmic rays in intergalactic space, and the possible effects of large scale regions of antimatter in the Universe. These discussions lack the discipline imposed by reliable experimental results and the more hardened experimentalists will look upon them with a degree of cynicism.

The second breakthrough—the discovery of γ -ray bursts from outside the Solar System—was not published until after this symposium, but it was thought to be sufficiently important for two papers on the subject to be in-

cluded in the report.

The final session of the symposium was a discussion of future trends in γ -ray astronomy. This discussion leaves one with the impression that all γ -ray astronomers are aware of the difficulties facing the subject but that some, at least, are optimistic. The low price of this report, its clear format and its quick publication combine to make it an attractive summary of the current state of this branch of astronomy.

R. R. Hillier

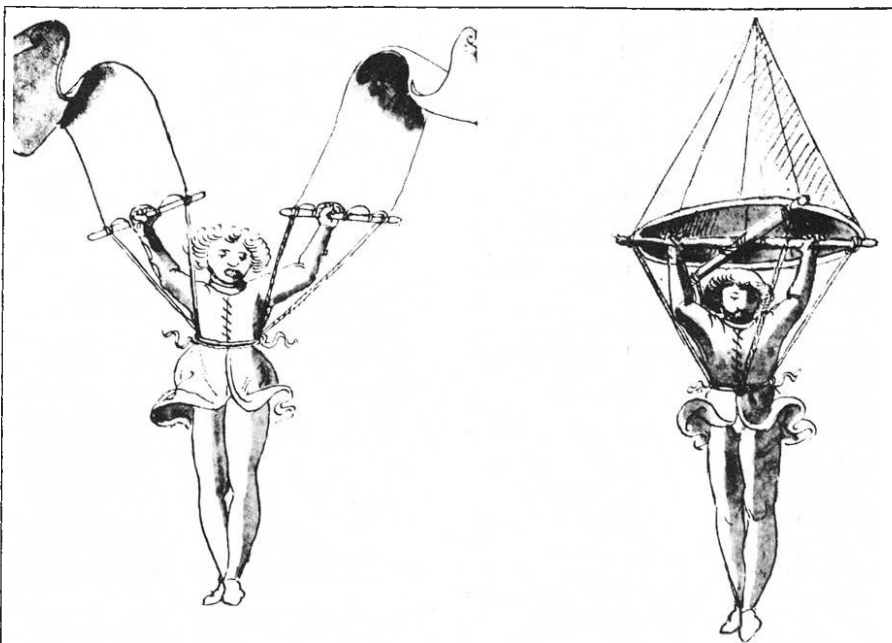
...and for arts students

Concepts of Contemporary Astronomy. By Paul W. Hodge. Pp. viii+547. (McGraw-Hill: New York, 1974.) \$9.95; £5.45.

Fundamental Astronomy. By Franklyn W. Cole. Pp. xiii+476. (Wiley: New York, 1974.) £5.30.

WITH deafening regularity new books on astronomy hit the librarians' tables, all aimed at that enormous amorphous bunch of students known as "non-science majors" who annually flock to American universities to take degrees in liberal arts and letters. One gets the impression that no American publisher worth his salt can produce the yearly book list without at least one *Introduction to Astronomy*, *Fundamental Astronomy*, *Modern Astronomy*, *Principles of Astronomy*, *New Horizons in Astronomy*, *Stars and Galaxies we Ought to Know and Love*, *Concepts of the Universe*, *The Universe and Beyond!*, *Mysteries of Space*, and so on.

The teaching of science to arts students is obviously a good thing.



Parachutes: optimism and progress. Anonymous sketches from the late fifteenth century. The first recorded drop (probably an entirely appropriate word) was made in 1783. From *Scientific Technology and Social Change*. (Readings from Scientific American.) With introductions by Gene I. Rochlin. Pp. 403. (W. H. Freeman: San Francisco, 1974.) \$6.95.

Science impinges constantly on everyday life and ought to be understood at least in part. Astronomy is the most accessible science to the arts student: after all isn't every thinking person a cosmologist? Even the child ponders earth, space and time, their beginning and end. And astronomy is central among the sciences, gathering knowledge and expertise from a myriad of scientific specialities. Both the significance and insignificance of man in relationship to time and the universe are clearly illustrated.

But how should the arts student be approached? Most of the books seem to regard him as a rather stupid, innumerate, failed scientist. Surely this student has a different outlook on life from scientists, an outlook bred on comparison and criticism and aesthetic judgement, a preoccupation with why as opposed to the narrower scientific how. Science should be able to learn as much from arts students as they do from scientists, and these books should help and encourage arts students to play their part in quenching the thirst for knowledge.

Both the books under review are good in their own way. Both are clearly written in a concise and ever-questioning style, continually leading the reader to grasp the enormities of the problems being tackled by astronomers and the many pitfalls on the way to understanding. Both books are lavishly illustrated with photographs of instruments, planets, stars and galaxies and, especially in Hodge's book, with thoughtfully designed simple line figures.

Each book considers basic astronomical concepts, the Solar System, stars and galaxies; however, Cole has slightly more emphasis on the Solar System, Hodge emphasising stellar evolution. Both books stress observation: Cole has an excellent appendix on observational astronomy for beginners; Hodge concludes each chapter with a fascinating list of suggestions for simple experiments and observations. Some of these require simple laboratory equipment or a small telescope or high powered binoculars but many require only impoverished equipment that can be put together at home.

Choosing between the two books I would pick Hodge's *Concepts of Contemporary Astronomy*: the best book on astronomy at this level that I have read for many years. Hodge has thought very carefully about the needs of the students he is writing for and gives them not only a knowledge of the nature of the universe but also an appreciation of the methods of science. But I still have a hankering to read an astronomy text written by an arts graduate. I wonder what they really make of it.

David W. Hughes

Sources of streams

Fluvial Processes in Instrumented Watersheds: Studies of Small Watersheds in the British Isles. By K. J. Gregory and D. E. Walling. Pp. iii+194. (Institute of British Geographers Special Publication No 6.) (Institute of British Geographers: London, February 1974.) £4.25; \$10.60.

THIS latest volume by the British Geomorphological Research Group reports the results of major process investigations in small catchments in Britain. The work involves contributions in four major areas: the origins and controls of run-off and of sediment and solutes. The objectives of the studies range from basic assessments of the volume and frequency of inputs and outputs to the basin, to formal testing of deductive models. For the most part, however, the work is empirical and much of it applied or capable of direct application. It should attract the attention of practising civil engineers as well as researchers.

Two dilemmas are brought out

clearly in the volume and referred to by the editors. The first is that within the basin there are important spatial and temporal variations in processes whose effects are integrated in sediment, solute and water yield at the output. Unfortunately, the output is where the monitoring of the basin is likely to be easiest and least expensive. In choosing small basins where one process tends to dominate, this dilemma has been partly avoided. Moreover, the work then becomes complementary to, rather than competitive with, that carried out by other research organisations. The second dilemma is that with the rapid development of the subject, model building alternately precedes and succeeds data collection for testing. The solution seems to be the closer integration of the two areas of research.

This is an important addition to research in geomorphology and perhaps to hydrology. Despite an over-imaginative use of the linear regression model, it indicates a very healthy state in a key area of concern in environmental research and management.

John B. Thornes

Earth science by numbers

Geomathematics: Mathematical Background and Geo-Science Applications. By F. P. Agterberg. Pp. xvi+596. (Developments in Geomathematics, vol. 1.) (Elsevier: Amsterdam, London and New York, 1974.) Dfl135; \$54.00.

THIS book surveys the mathematical methods and statistical techniques used in the study of data obtained from various types of physical and chemical sampling of rock and rock strata at the Earth's surface. It is not, as its title might imply, a comprehensive survey of the mathematical methods used in general geophysics. Rock samples, and geological data generally, are characterised by very great variability and so it is not surprising that this book concentrates on probability and statistics.

The book is unique in that these statistical methods are illustrated by many examples drawn from geology. In a way it is strange that it was biology rather than geology that provided the stage for the development of modern statistical methods. Lyell had in an appendix to his *Principles of Geology* divided the Tertiary into four periods on the basis of the proportion of species found in these rocks living today. But on the whole the field geologist, unlike the agriculturalist, did not find it easy to record in a simple numerical form the various pieces of information he collected and perhaps remained unconvinced of the value of doing so. It was of course the long series of carefully

marshalled numerical data at Rothamstead that gave the late Sir Ronald Fisher the impetus he needed to lay the basis of modern statistics.

It is, however, interesting to note that the impetus from the development of a statistical theory of variation in two and three dimensions resulted from the accumulation of data on the distribution of cross bedding directions and palaeomagnetic data respectively. All this is now changed and this book will be of great value to the geologist.

S. K. Runcorn

Statistical Methods for the Earth Scientist. By Roger Till. Pp. 154. (Macmillan: London and Basingstoke, 1974.) £5.00, boards; £2.50, paper.

THERE are few really readable books on statistics in general and even fewer aimed specifically at the earth scientist, but Dr Till is to be congratulated in joining a select and much needed band. The book is intended as the basis for a 40 hour course in statistics for undergraduate or postgraduate students with no previous background in statistics, and only simple algebra. It is a small volume—9.5 by 6 inches—with a content ranging from the basic philosophy of statistics up to analyses of variance, regression and some non-parametric tests. These are covered in a comprehensible way with examples drawn from mainly sedimentological and geochemical sources, although care has obviously been taken to include at least

one example from each of the main branches of earth science.

The book should form an excellent basis for a very elementary course (even less than the prescribed 40 hours) as it not only covers the basic concepts but also gives ample warnings of the standard pitfalls into which so many senior earth scientists have fallen—and are still falling. To quote one example, not used in the book, radioactive isotopic dates invariably include their precision, that is, the repeatability, yet most earth scientists still take these figures as measures of accuracy, at least until they conflict with their own ideas.

Other credits are good references and the avoidance of that frustrating phrase "it can be shown that"; instead Dr Till refers the reader elsewhere, usually to Yule and Kendall, for a more rigorous explanation but even that is rare. The publishers also deserve credit as the book is well produced and designed, although certain figures (for instance, 5.5, 5.6 and 5.7) are really too big and, if reduced, would not only have saved paper but would also have improved the appearance of the book.

The index covers only statistical subjects, and it might sometimes be necessary to find out where porosity or grain size is considered. But the only real fault is the scope: the range of analyses covered is much too short for the cost of the book and it would also have been a better buy if more statistical tables had been included.

I hope that Dr Till will be encouraged to expand this introductory text to include, for example, much fuller consideration of directional properties so that this book, if still sold at the same price, could become an essential text at the start of any earth scientist's career. But at the moment it does not go far enough.

D. H. Tarling

Model methods

Analytical Methods in Planetary Boundary-Layer Modelling. By R. A. Brown. Pp. xii+148+5 plates. (Adam Hillger: London, June 1974.) £8.00.

THE scope of this book is narrower than one might guess from the title. It deals primarily with a restricted topic in the theory of the Earth's atmospheric boundary layer, namely the mean velocity distribution in stationary, horizontally homogeneous, and neutrally stratified conditions. Particular emphasis is placed on the matching of an upper Ekman layer with a surface layer unaffected by the Earth's rotation. Concluding chapters consider the effects of non-neutral stratification and non-stationarity, but the treatment is very superficial. There are also two chapters on the instability of Ekman layers and the relationship between that and meteorological observations; this

is the best part of the book but its connection with the rest is not made clear.

For whom is this book intended? The author offers no indication, and the level of difficulty is so variable that one cannot guess. Who is the mysterious reader who can comprehend the equations of fluid motion with no derivation and little explanation, but who requires an elementary explanation of the procedures of dimensional analysis; who is already familiar with the properties of geostrophic flow, but not with the basic derivation of centripetal and Coriolis accelerations; who knows what is meant by "the eddy flux of potential heat", but who can usefully read a most superficial summary of the properties of thermal convection?

Meteorology students might benefit from a text leading up to current views of the atmospheric boundary layer through explanation of the now classical topics of Ekman layer theory and micrometeorology; synthesis of those topics is not so commonly discussed. Brown's book is, however, neither comprehensive enough nor lucid enough

to serve that purpose. The derivation of the well known logarithmic profile, for instance, would be quite incomprehensible to anyone not already familiar with it.

Errors in the equations and algebraic expressions are sufficiently numerous to provide further reason for not recommending the book to readers unfamiliar with the subject. Most of the errors are probably misprints (although there are too many of them for this to be acceptable as an excuse). But there are incorrect signs on pages 49 and 79 which invalidate the subsequent algebra; and equation (3.19) is, I suspect, a misprinted version of an incorrect equation.

Research workers in fluid dynamics and meteorology may occasionally find the book a useful source on those topics for which the information is not gathered together elsewhere. But this purpose could have been fulfilled more effectively and economically by a review article on, for example, 'Ekman layers in meteorology'.

D. J. Tritton

There's still no place like Holmes

Earth. (A series of books in Geology.) By F. Press and R. Siever. Pp. xi+945. (W. H. Freeman: San Francisco, July 1974.) £6.60.

THE authors of this book have impeccable qualifications for writing it, both as active research scientists and as university teachers. They say that it is intended for students who have had no previous college science courses; and they express the hope that it is as up to date as a current meeting of the Geological Society of America (it was finished in December 1973) and that at the same time, it is as understandable as today's newspaper. These intentions are commendable and the task they have set themselves is difficult; it is interesting to see how they tackle it.

The level throughout the book, which is splendidly produced and illustrated, is such that after reading it the diligent student should experience no difficulty with specialist papers in *Scientific American*. Plate tectonics figures large and early. The first 150 pages—"The earth as an historically evolved body and how we study it"—serve to introduce the vocabulary. They start with an account of the origin of the Solar System, which I found interesting because I knew nothing about it, and finish with a long chapter on rocks and minerals. Part II covers the traditional field of physical geology. Part III introduces the fields of petrology and geophysics and this section is written at a marginally higher level than the others; for example, a chapter on the

formation of igneous rocks touches on Bowen's reaction series and on the problem of the origin of granite batholiths. I wonder, however, whether the 27 pages on plutonism and metamorphism really equip the reader to dip into F. J. Turner's book *Metamorphic Petrology* which is quoted in the bibliography.

How, then, does the book live up to its authors' intentions? Trying to cope with a readership which is assumed to be entirely without any scientific education has made the book unmanageably long. Was it necessary to devote three pages (115–118) to defining words like topography and contour? The need to start the chapter on weathering with the very beginning of inorganic chemistry makes it impossible to discuss the peculiarities of the weathering of rocks at all. The desire to encompass past present and future in one large sweep reminds me of the phrenetic enthusiasm of geophysicists running an open day in the laboratory. For example, in chapter 3 ("How we find out about the earth") we have a photograph of a Benioff strain seismometer, a section on experimental petrology and a 'box' about Cavendish's 1798 determination of *G*, together with 15 other topics all introduced in the course of 22 pages. The book is certainly not dull, but I wonder whether the American college student has the stamina to read its 945 pages. I do know that the English girl studying for 'A' levels on whom I tried it, had not. In this field Holmes remains ancient but unbowed.

D. H. Matthews

Broad spectral lines

Spectral Line Broadening by Plasmas. By Hans R. Griem. Pp. xiv+408. (Pure and Applied Physics: A Series of Monographs and Textbooks, vol 39.) (Academic: New York and London, May 1974.) \$31.50; £15.10.

AN earlier book by this author, entitled 'Plasma Spectroscopy', has served for many years as a reference source for spectroscopic research in experimental plasma physics and astronomy. The popularity of that text, written in 1964, can be gauged by its high demand in departmental libraries, and the frequency with which it is encountered in spectroscopic laboratories. The new monograph treats a more specialised topic: spectral line-broadening mechanisms for neutral and ionised atoms in quiescent plasmas, and in nonequilibrium plasmas which exhibit suprathermal electric field fluctuations. It thus expands and updates chapter 4 of the earlier book, and similar theoretical reviews of the same vintage by Baranger, Van Regemortel, Traving and others.

Professor Griem introduces his subject with an admirably concise survey of the foundations of Stark broadening theory, and the derivation of the quasi-

static and impact approximations. Detailed examples of practical calculations using these two extreme, but complementary, approximations are then given; these are followed by a discussion of various intermediate approximations (phase integral, relaxation, dynamical and one-electron) for situations in which the frequency shift relative to the unperturbed line frequency is neither much less nor much greater than the characteristic frequency for single, binary, collisions with charged perturbers. Several other characteristic frequencies may enter the general problem. Thus, magnetic field and plasma correlation effects (Debye shielding, plasma polarisation shift and satellites caused by plasma wave fields) are selected and discussed in the final sections of a chapter which comprises some 40% of the total text. (In his preface the author wisely, though modestly, advises the experimentalist that a 'minimum theory' approach has been adopted.)

The next fifty or so pages constitute a highly selective, and therefore most interesting, critique of experiments which have provided important checks on overall accuracy, and validity limits to the various theoretical approximations. Most definitive experiments have been made on laboratory plasmas with electron densities of 10^{13} – 10^{19} cm $^{-3}$, and temperatures of 2×10^3 – 2×10^7 K. This density regime is much more restricted than that of current practical and computational interest, which ranges from approximately 10^3 cm $^{-3}$ (H II region) to 10^{26} cm $^{-3}$ (stellar interiors and laser fusion). Similarly, the available temperature range is still one or two decades less than that of thermonuclear interest. The experimenter should note how frequently density gradients have caused real complications in the interpretation of line shapes, even when some other, independent, density measure is also available. Relatively few line-broadening analyses seem to have been reported for heavy-ion hydrogenic lines and for lines in third and higher spectra, although plasma polarisation effects can be of particular significance to the latter spectra, because reliable corrections are needed to deduce the corresponding energy levels of isolated atom. At sufficiently high densities, neutral helium emits lines with forbidden components, involving transitions in which the angular quantum number does not change by unity.

More generally, the electric fields associated with suprathermal plasma waves will modify the line shape, and can excite satellites symmetrically disposed about the position of forbidden components. The third chapter, describing key experiments, therefore con-

cludes with a discussion of recent attempts to probe the intensity and orientation of turbulent electric fields, by measuring the intensity of sidebands on H $_{\beta}$ profiles in two orthogonal directions, and by analysing the polarisation of the emitted spectrum. Satellites on He I lines associated with electron and with ion plasma oscillations have also been identified; in some of these measurements wave energies three to four orders of magnitude above thermal levels were inferred. A particularly pretty example of the sophistication of present theory and experiment is illustrated in Fig. 28, in which Cooper and Hess have resolved fine structure caused by the Zeeman effect on a forbidden line, and on plasma satellites, by comparing π and σ polarisations.

For the many of us who are users rather than specialists in the subject, the fourth and final chapter provides a very convenient summary and conclusion to the text. It outlines various practical applications, including determinations of plasma density and temperature, and the investigation of strongly excited collective modes in nonequilibrium plasmas; stellar emission, radio frequency lines from galactic regions, and the importance of the wing shapes of resonance lines to opacity and radiative transport calculations are briefly discussed. It also serves to introduce numerical data tabulated in some hundred pages of appendices. (Such information should be augmented in the future by information collated at a data centre recently established at the National Bureau of Standards.)

One valuable service provided by a book of this sort is the correction of errors which have crept into the literature; such errors are discussed, for example, at equations (207), (394), (395) and on pages 150, 176, 204 and 215 (references 145, 59, 258 and 38, respectively.) Presumably to avoid further errors, and to reduce costs, many of the tables are reproduced as computer printout; in the review copy some of these were difficult to decipher (for example, Table 3), but this constitutes only a minor criticism concerning the production of a book which is attractive to read, well organised and realistically priced. Professor Griem and his research school have made very significant contributions to this subject, and the book should be well received at a time when a topical interest in laser-fusion plasmas should give the subject a fresh lease of life. In this connection, it may be noted that the effects of extremely high densities, in which the plasma electrons obey Fermi-Dirac statistics, are not discussed explicitly.

Ian Spalding

The Athlone Press UNIVERSITY OF LONDON

Island Survivors: The Ecology of the Soay Sheep of St Kilda

(ed.) P. A. JEWELL, C. MILNER
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'A pioneer work which is likely to remain essential for . . . those who nowadays have an interest in the quality of the air we breathe.' *Nature*

55 figs.

£6.25

Mathematical theory of elasticity

Introduction to Rational Elasticity. (Monographs and Textbooks on Mechanics of Solids and Fluids.) By C. C. Wang and C. Truesdell. Pp. xii+556. (Noordhoff: Leyden, 1973). Dfl. 130.

FOLLOWING the precedent created by his joint work with Noll in presenting the non-linear field theories of mechanics Truesdell has now collaborated with C. C. Wang to bring together recent work in the mathematical theory of elasticity. As Truesdell points out in his introduction, there has been a dearth, until recently, of rigorous theoretical work on the existence and uniqueness of solutions to problems of elastic materials. With completely new techniques in pure mathematics becoming more generally understood, however, the stage is set for advances in many branches of applied mathematics. If this book inspires pure mathematicians to embark upon the application of rigorous mathematics to problems in applied mathematics and, in particular, elasticity then the authors' aims will have been vindicated and the colossal price of the book perhaps justified.

For those readers not familiar with the recent advances in pure mathematics, the book opens with a chapter which present the concept of a finite dimensional vector space, the concept of a manifold, and a section on Lie groups. This chapter is, as the authors admit, an outline of ideas, and many readers will need to study more deeply the topics covered before feeling confident to proceed further. Chapter II considers the more general subject of continuum mechanics, of which elasticity is one aspect. It is suggested that a reader whose interest lies solely in elasticity may ignore this chapter—that would be very unwise because in chapter II is the development of the ideas of constitutive relationships upon which modern elasticity theory is based. These relationships are further developed in the context of elasticity in the third chapter, which also contains a number of the inequalities satisfied by an elastic body, together with an introduction to the uniqueness and stability of solutions of elastic problems.

Homogeneous and inhomogeneous bodies are studied in the following two chapters, and chapter VI contains a discussion of wave propagation. In that chapter the less mathematical reader will recognise many familiar results and it could be argued that a good introduction to rational elasticity might be obtained by a superficial reading of chapters IV–VI before embarking upon a detailed study of these and

the earlier sections of the book.

The final chapter presents a unified account of the present state of knowledge of the existence, uniqueness and stability of the boundary-value problems of elasticity. This is the climax of the book for the mathematician and should leave him in no doubt that there are still many problems worthy of his skilled attention.

A. H. Craven

Crystal behaviour

Dislocations and Plastic Deformation. By I. Kovacs and L. Zsoldos. (International Series of Monographs in Natural Philosophy, Vol. 60.) Pp. xii+343. (Pergamon: Oxford and New York, February 1974.) £3.50.

THIS book aims to provide an introduction to dislocation theory, followed by an account of the mechanical properties of crystals, including the yield stress, work hardening, creep, and effects of heat treatment. It covers much the same ground as Cottrell's famous book *Dislocations and Plastic Flow in Crystals*, but attempts to update and enlarge upon that work, now 20 years old. It is a difficult task, for the boundary between dislocation theory and plasticity is as much of a no-man's land as ever, and Kovacs and Zsoldos can only list the conflicting theories of work hardening without leaving the reader much wiser; a student will find this chapter heavy and demoralising.

It is unfortunate that the treatment is so exclusively devoted to tensile stress-strain curves of pure face-centred cubic metal single crystals: a wider discussion of the experimental observations would be rewarding. Another general criticism of the book is that it omits many recent developments: there is no mention of Foreman and Makin's computer experiments on yielding, which have greatly clarified the subject; no mention of weak-beam measurements of stacking-fault energy and no mention of diffusion creep. Finally, the book contains a number of errors, perhaps the most glaring being the expression for the energy of a straight dislocation on page 48, which leads to erroneous expressions for the line tension on page 64, and this in turn, together with the omission of weak-beam results, leads to a rather unbalanced assessment of the current value of stacking-fault energies.

In spite of these defects, the book is more comprehensive and up to date than any now available, and provided the graduate student has read Cottrell's book referred to earlier, this book can be used as an introductory text in a field where few are available.

L. M. Brown

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Arthur W. Ham M.B. F.R.S.C. D.Sc. *Seventh Edition*, 1974. 1,024 pages, 690 illustrations. Lippincott, £12.40.

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Carl Gans. 1974. 288 pages, 120 illustrations. Lippincott, cloth £6.25, paper £3.00.

Handbook of Microbiology

Edited by Allen I. Laskin Ph.D. and Hubert Lechevalier Ph.D. *Condensed Edition*, December 1974. 944 pages, 150 illustrations. CRC Press, £10.00.

Methods for cool operators

Low Temperature Laboratory Techniques: The Use of Liquid Helium in the Laboratory. Second edition. By A. C. Rose-Innes. Pp. 255. (English Universities: London, 1973.) £3.95.

THIS is a new edition, brought up to date (although no references are later than 1971) and expanded with sections on developments since 1964. The main sections are concerned with the temperature range above 1 K, with coverage of the handling of helium, cryostats, and the design of apparatus, and the measurement and control of temperature. Towards the end are chapters on the use of ^3He (including dilution refrigeration, but not Pomeranchuk refrigeration) and on temperature measurement below 1 K.

The advice given is at an unusually detailed practical level, often to such an extent that every dimension and technique seems to carry a personal guarantee. The numerous figures reinforce this feeling by their directness and simplicity. I can see the value of this approach, particularly for the beginner who often has to take responsibility for the lowlier aspects of research without the author's experience. Inevitably, with such a laboratory-workbook format there are places where the reader may wish to add, remove or alter, but on the whole it is a useful collection of highly practical tips. The data tables are also helpful.

There are a number of unnecessary variations of notation (for example He^3 on p. 193; helium³ on p. 194; ml on p. 22; cm^3 on p. 23); misprints (I enjoyed "owl temperature" on p. 99 and "aneroid" on p. 114 among others) and unnumbered equations. Generally, though, appearance and visual clarity are good.

D. S. Betts

Nuclear structure

Theoretical Nuclear Physics. Vol. 1: Nuclear Structure. By Amos de Shalit and Herman Feshbach. Pp. xxviii + 979. (Wiley: New York and London, June 1974.) £14.60.

THE appearance of this volume is a notable event for nuclear physicists and yet another reminder of the loss to the scientific community caused by the untimely death of Amos de Shalit. It is the first of two volumes covering essentially the whole of nuclear physics; this one is devoted to nuclear structure and a forthcoming volume will cover nuclear reactions.

The authors aimed to cover all the important ideas as clearly as possible,

and in a way that will inspire as well as instruct, and they have succeeded brilliantly. A brief summary of the contents will indicate the scope of this encyclopaedic work.

The first chapter is an introductory review of nuclear physics, dealing with nuclear sizes and masses, forces and energies, and also with nuclear systematics, regularities in nuclear spectra, electromagnetic properties of nuclei, high energy scattering and reactions and associated topics. Subsequent chapters are devoted to nuclear models, starting with the simplest that likens the nucleus to a Fermi gas. There are sections on the Weisäcker mass formula, one and two-particle densities, coulomb energies and the nucleon-nucleon force and nuclear stability. Next comes a chapter on nuclear matter, basic to the understanding of nuclear properties. The independent-particle and independent-pair approximations are described, together with the Bethe-Goldstone equation and its solution.

Two chapters are devoted to the independent particle and shell models. It is shown how the single-particle shell model with either an harmonic oscillator potential or a more realistic rounded and finite potential is able, provided a spin-orbit term is included, to account for many basic features of nuclear structure, in particular the magic numbers and the spins, parities and magnetic moments of nuclear states. The model works best for nuclei near closed shells, but it is also shown how it may be extended to nuclei with several particles outside closed shells, and its success is illustrated by examples. The Hartree-Fock self-consistent field theory is developed and applied to closed and open-shell nuclei. The method of classifying nuclear states with the help of the concept of isospin is described and illustrated by examples, using various coupling schemes. There are also sections on particle-hole configurations, fractional parentage coefficients and configuration mixing in many-particle systems.

Away from closed shells it becomes increasingly difficult to use the shell model to describe nuclear states because of the very large numbers of possible configurations, and for such nuclei the collective models are often more appropriate. In such models many nucleons are assumed to move together, either as a rotation or as a vibration of the whole nucleus. With an appropriate collective Hamiltonian it is possible to calculate many of the properties of such nuclei.

The theory of multinucleon systems is considered next, with a detailed account of the Hartree-Fock theory, the random phase approximation and the free quasi-particle system, all using

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the second quantisation formalism. There are sections on the pairing interaction, the linked-cluster expansion, the Brueckner-Hartree-Fock theory, the local density approximation, the pairing-plus-quadrupole interaction and the microscopic theory of valence forces.

The last two chapters are devoted to electromagnetic transitions and weak interaction phenomena. After a description of the nuclear electromagnetic current and the quantised electromagnetic field the selection and sum rules governing electromagnetic transitions are obtained and illustrated by examples. The section on weak interaction begins with the theory of β decay and goes on to consider different types of transitions, orbital electron capture, antineutrino absorption, electron-neutrino angular correlation, the helicity of the neutrino, muon decay, vector currents, forbidden beta decay and absorption of muons by nuclei.

This brief summary of the main topics can do little to indicate the wealth of detail and comprehensiveness of the coverage of the book. It is difficult to think of any important aspect of nuclear structure physics that has not been covered, and the vast range of topics is welded into a coherent whole.

The treatment is generally at the graduate student level, and requires only a general familiarity with nuclear physics such as may be obtained in an undergraduate course. It will also serve as a valuable reference book for undergraduates and a handbook for professional nuclear physicists.

P. E. Hodgson

Denial of quantum physics

The Interpretation of Quantum Mechanics. By Michael Audi. Pp. xiv+200. (University of Chicago: Chicago and London, 1973.) £5.75.

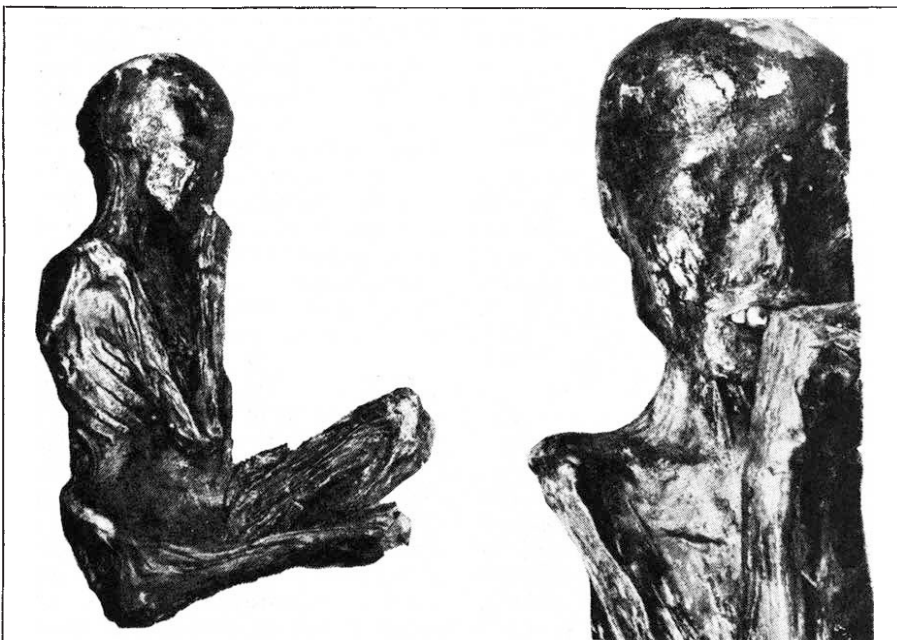
THE main purpose of this book is to show that the classical conception of a particle, particularly the attribution of simultaneous position and momentum to a particle, can be carried over to quantum mechanics. The core of the argumentation runs as follows: classical mechanics can be separated into two parts. First, a material body can be represented as a collection of particles, each particle having at all times an exact position and an exact momentum and thus travelling in a continuous path in space. Second, the path of a particle is determined by laws of motion. In quantum mechanics, however, there exist no laws of motion for atomic particles, and the indeterminacy of observations is due to the unpredictability of the paths of material atomic particles.

This means, of course, that it is erroneous to ascribe any wave property to a material atomic particle. Conversely, the particle property of photons is denied. A photon is considered to be oscillations in the electromagnetic field, and all the experimental evidence of the corpuscular character of electromagnetic radiation is explained as "discrete interaction properties" of the electromagnetic field with matter. Obviously this amounts to a

total rejection of the complementarity of quantum physics.

By means of his thesis about the unpredictability of the (postulated) paths of material atomic particles, the author is to some extent capable of protecting his classical conception of an atomic particle from the experimental facts in quantum physics. But on page 106 it is rightly stated that any satisfactory interpretation of quantum mechanics must provide an explanation of crystal diffraction phenomena. Now the classical particle concept will come to its crucial test, the reader thinks. But instead of introducing in some statistical way the postulated particle trajectories, the incident beam of particles is represented in the usual quantum mechanical way by a plane wave function, which is then perturbed by a periodic potential representing the crystal. Though it is indeed an interference phenomenon which the author sketches in these calculations, he does not like to say so. Multiple slit experiments are treated along the same line, and the argument ends up with the rather cryptic statement that each particle in the beam passes through one particular slit, but, nevertheless, more than one slit is acting on the particle.

The parts of the mathematical scheme of quantum theory involved in the argument are far too sketchily presented and several equations are blurred by misprints. Also, the author seems to minimise the significance of points which oppose his basic position, and in some cases he simply leaves out such points. For instance, the Copenhagen interpretation of quantum mechanics is said to be inconsistent because it claims that quantum physics contains classical physics as a limiting case and yet it does not consider atomic particles as classical particles. One should expect, then, that the author would examine very carefully the correspondence between the two fields of experience. On pages 34-35 he claims to have sketched a derivation of the classical Hamilton-Jacobi equation from Schrödinger's equation in the limit where the quantum of action can be considered to be vanishingly small. It really cannot be called a derivation because it involves equations which are mathematically objectionable, and, furthermore, the most important result in this connection, namely that the wave packet representing the atomic object moves according to the laws of classical mechanics, is left out. Yet this result shows that the complementary behaviour of atomic objects displayed through interference phenomena and particle phenomena is fully compatible with the classical particle concept, applying, of course, only to observational situations where the quantum of action can be neglected.



Two views of 'Little Ape', found in Salts Cave in Kentucky. The surrounding region is well known for the richness of its archaeological remains. From *Archaeology of the Mammoth Cave Area*. (Studies in Archaeology.) Edited by P. J. Watson. Pp. 21+255. (Academic: New York and London, July 1974.) \$16.00; £8.00.

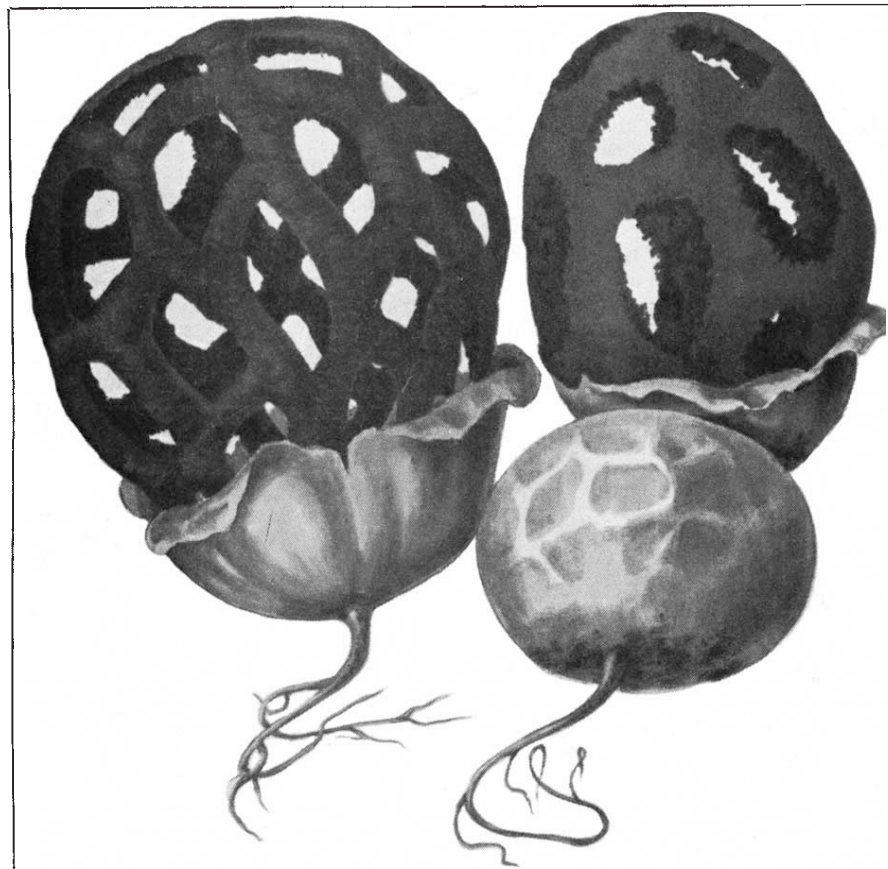
Gradient liquid chromatography

Gradient Liquid Chromatography. By C. Liteanu and S. Gogan. (Elli Horwood Series in Analytical Chemistry). Pp. xii+358. (Ellis Horwood; Chichester, Distributed by John Wiley. Halsted Press: New York, July 1974.) £10.50.

THIS work reviews the principles, practice and application of liquid chromatography using various types of gradients in eluent and stationary phase composition, and in temperature. The review is comprehensive up to 1969 or 1970 but the authors have unfortunately failed to react fully to the recent development of high performance liquid column chromatography and the practical parts of the book are somewhat dated although still useful as a basis for development.

In reviewing the theory the authors are too uncritical and by quoting numerous mathematical results, often without adequate explanation, they have failed to explain the principles of the subject as clearly as they could have. This is unfortunate in an otherwise useful work for it limits its value to practising analytical chemists who badly need a clear and simple exposition of this important topic.

John H. Knox



Clathrus cancellatus. One of hundreds of illustrations in *Mushrooms and other Fungi*. By Augusto Rinaldi and Vassili Tyndalo. (Translation by Italia and Alberto Mancinelli.) Pp. 333. (Hamlyn: London and New York, November 1974.) £6.95.

The author's thesis about atomic particles means, in fact, the rejection of quantum physics itself. If the reciprocal limitations in the applicability of the space-time concepts and the momentum-energy concepts expressed by Heisenberg's indeterminacy relations are not fundamental, but can eventually be overcome by improvements in observational techniques and theoretical methods, then there exists no universal quantum of action and no quantum physics. **T. Bergstein**

Molecular chemistry

Internal Rotation in Molecules. Edited by W. J. Orville-Thomas. Pp. xvii+606. (Wiley-Interscience: New York, 1974.) £13.50.

THE last thirty years have seen considerable activity in the study of internal rotation and related ring puckering motions in molecules. Orville-Thomas' extensive volume is timely, containing a chapter on all of the main techniques and the results which can thus be obtained. It is, unfortunately, an edited volume with separate authors for each chapter, a scheme which leaves a few gaps and introduces some duplication and variation of style. It is a pity that the editor

did not impose a *fiat* on the question of units for barrier heights: the choice of kcalorie mol⁻¹ dominates over the more favoured kJ mol⁻¹ by about 2:1. It is perhaps inevitable, but many of the chapters take space to introduce fundamentals, so that the volume includes unnecessary introductory accounts of nuclear magnetic resonance (n.m.r.), gas microwaves, electron diffraction and acoustics.

Gas microwaves provide the single most valuable technique for gaining information about the gas phase and the topic is covered by N. L. Owen in a fairly short but effective chapter. Electron diffraction, described by A. H. Clark, is valuable chiefly for identifying the dominant conformation, and in some cases for evaluating the trans-gauche ratios. Acoustic work, described by S. M. Walker, covers the kinetics of the transformation, although the size of dispersion also indicates energy and volume differences between the interconverting systems. There is also a helpful chapter on theoretical work, covered by A. Veillard, from which it is clear that elaborate *ab initio* calculations can evaluate the barriers effectively although it is not easy to extract simple qualitative information about the origin of the

barrier from such complex wave functions. Simpler functions are ineffective in predicting the barrier heights.

One of the difficulties in this field is the major influence of solvents, and in liquid phases the effective barriers and potential minima may be quite different, a point strongly emphasised by R. J. Abraham and E. Breit-schneider. The regular tools for liquid-state studies include n.m.r. and dielectric methods.

Other chapters complete the picture which, overall, amounts to a great deal of work by many research workers. There is sufficient knowledge to allow most unknown barriers to be inferred by analogy and interpolation, at least in the organic field. References to completely inorganic molecules are very sparse and there did not seem to be any reference to the intriguing motion which makes all the fluorine atoms indistinguishable in PF₅ at higher temperatures. A formulae index would have added greatly to the usefulness of the book.

Clearly, most specialist science libraries will need this volume, but the overall picture is neither sufficiently clear nor sufficiently intriguing to warrant individual purchase.

D. H. Whiffen

Connective issues

The structure and Biochemistry of Cartilage. By A. Serafini-Fracassini, and J. W. Smith. Pp. vii+236. (Churchill Livingstone: Edinburgh and London, 1974.) £8.00.

THIS book is a very readable account of the biochemistry of connective tissue components, and of the structure and morphology of cartilage. It provides a good background to the biochemistry of connective tissue and gives an excellent introduction to cartilage morphology with particularly detailed accounts of the epiphyseal plate and calcification. In those topics it is a major advance upon existing texts.

The first half of the book gives a detailed account of the structure of elastin, collagen and the glycosaminoglycans. The subject is treated thoroughly and would do justice to a more general text on connective tissue biochemistry. Much of the information is contained in numerous tables which compare chemical compositions and amino acid analyses, and list physico-chemical properties. This concise way of presenting data is backed by a text that is informative and to the point, which greatly enhances the value of the book to the general reader.

The molecular biology of connective tissue macromolecules is probably the most rapidly developing field of research relating to cartilage, and the inevitable delay between the finish of literature searches and publication has produced some points at which these sections are now out of date. For example, there is little emphasis on the different genetic species of collagen present in different tissues. The distinction in composition between cartilage collagen $[\alpha I(I)]_3$ and skin-bone collagen $[\alpha I(I)]_2 \alpha_2$ is described, but their significance as gene products that are tissue specific is not discussed at all. Similarly, new information on the organisation of proteoglycans has shown that they aggregate by binding to hyaluronic acid, forming very high molecular weight complexes within the cartilage matrix. The staining of proteoglycans in cartilage sections for electron microscopy has, therefore, more recently been reassessed taking this into account. The problem that information becomes rapidly out of date is always present to a greater or lesser extent in scientific reviews but in this instance it does not detract from the usefulness of the large amount of additional information presented.

The second half of the book is concerned with the morphology of different types of cartilage. The transition from biochemistry to morphology is bridged by two chapters in which tech-

niques of studying connective tissue components with both the optical microscope and electron microscope are appraised critically, and the general ultrastructure of cartilage is reviewed. The remainder of the book then deals in some depth with the development and structure of the epiphyseal plate and with the calcification of cartilage. It finishes with shorter sections on articular cartilage and elastic cartilage. The chapters on the epiphyseal plate are particularly worthy of mention as they give a comprehensive account of its development, structure, nutrition, the formation of matrix, and the presence of lysosomal enzymes during the subsequent calcification of the tissue. It is difficult to find these topics described in such detail elsewhere.

Cholinergic transmission – from Russia

Acetylcholine: an Approach to the Molecular Mechanism of Action. By M. J. Michelson and E. V. Zeimal. Translated from the Russian by E. Lesser and Mira Lesser. (International Series of Monographs in Pure and Applied Biology. Division: Modern Trends in Physiological Sciences, vol. 38) Pp. xviii+241. (Pergamon: Oxford and New York, December 1973.) £8.50.

INTEREST in the application of techniques of molecular biology to the study of drug-receptor interactions, in particular at the cholinergic synapse, has grown rapidly in recent years. So the advent of a book claiming to discuss the "molecular mechanism of action of acetylcholine" would seem to be of vital and topical concern. But the book fails to generate the interest promised by its title. That may be partly because the authors concentrate much of their attention on the more classical "chemical-pharmacological" approach of determining structure-activity relationships; but it may also reflect the fact that, despite the initial advance afforded by success in the isolation of the cholinergic receptor, further progress in elucidating the molecular events whereby acetylcholine produces its effects has been disappointingly slow.

Nevertheless, the book includes much material of interest to students of cholinergic transmission. The authors describe work carried out by themselves and a variety of colleagues in Russian laboratories over the past 30 years. The reference list shows that most of this work has been published only in Russian, and may therefore be new to English speaking readers.

There is an initial introductory chapter on the electrophysiological and biochemical functioning of the cholinergic synapse, with an interesting

The book, primarily a text on cartilage, can perhaps be criticised for the allocation of excessive space to connective tissue biochemistry. Much of the research on the structure of elastin and collagen does not relate directly to cartilage. The book also lacks any attempt at a systematic account of the various types of mammalian cartilage or indeed any discussion of phylogeny, and for these reasons it cannot be regarded as a comprehensive work. The general standard of presentation is, however, very high. There is an abundance of illustrations and a particularly large number of electron micrographs. Each chapter also contains an extensive bibliography which enhances its value as a reference work.

T. E. Hardingham

account of what seems to be the first recording of the endplate potential, by Ginetsinsky and Michelson, in 1935. There follows a brief but informative discussion on the current knowledge of the chemical structure of cholinergic receptors. Again it is pleasing to read of evidence obtained by Turpaev in the early 1960s for the presence of sulphhydryl groups in the acetylcholine receptor, which anticipated the experiments of Karlin and his colleagues.

Theories of drug-receptor interaction are assessed well in the third chapter but the mathematical treatments are unnecessarily complicated by the avoidance of conventional symbols. The following three chapters deal essentially with results obtained from comparing the activities of a variety of acetylcholine analogues on both the acetylcholine receptor and acetylcholinesterase, from which the authors attempt to make deductions about the active centres of both molecules, and about the arrangement of receptors (or subunits of the receptor) on the postsynaptic membrane. Unfortunately, the treatment is lengthy and repetitive and the derived information is scanty and, at best, equivocal.

The final chapter, on non-synaptic cholinergic receptors, draws together data from a variety of tissues including foetal and denervated muscle, autonomic ganglia, Renshaw cells and *Aplysia* giant neurones, and fills in many of the gaps left in previous chapters. Once again, there is a surprising reference to results obtained by Ginetsinsky and his colleagues on foetal muscle some years before similar observations were made in laboratories outside Russia. It is to be hoped that publication of this book in English (an excellent translation) is a sign that such failures in communication will be less frequent in the future.

Vasanta Srinivasan

Primates: swinging through the trees . . .

Primate Locomotion. Edited by Farish A. Jenkins, jun. Pp. xii + 390. (Academic: New York and London, January 1974.) \$34.00; £16.30.

THE disadvantages of the current tendency to publish compilations of invited articles on specialised subjects are numerous. Though books of this kind have undoubted advantages, common to convenience foods and package holidays, they lack the combination of authoritative overview and synthesis of the single-author volume. One of their most serious weaknesses is that, being invited articles, they escape the wholly desirable quality control that anonymous referees and a critical editorial board can impose. Furthermore, the incautious reader may suffer in a different way when he discovers that only some of the articles are really new. Too often their substance, if not their dressing, has already been published elsewhere.

The principal fault of *Primate Locomotion* is the lack of overview and synthesis. With two or three exceptions the articles are new. They are generally of a high standard, lively, well presented and illustrated. It is apparent that currently at least two major problems are engaging the interest of

workers in the fields of anatomy, primatology and primate palaeontology. One of these—the arboreal ancestry of primates—is long overdue for resurrection; the other—brachiation and human ancestry—is a prime candidate for interment.

Cartmill sets the scene for the former in his discussion of the relative adaptive values of the clawed paw and the prehensile hand for arboreal life. He asks fundamental questions and supplies convincing answers illustrated by a wealth of comparative data from other mammalian groups. Too little attention has been paid in the past, as in this otherwise excellent chapter, to the sensory aspects of hand function. A hypothesis of the primacy of arboreality in the evolution of monkeys and apes is incomplete without consideration of the role of the peripheral sense organs.

Chapters from Farish Jenkins on former in his discussion of the relative locomotion of treeshrews, by Szalay and Decker on the evolution of the primate tarsus and by Walker on the locomotion of present day prosimians, pursue this theme. Jenkins's paper, as becomes an editor's contribution, is particularly sound and lacks any element of witch-hunting which diminishes the stature of some of the other articles. I particularly like Jenkins's final sentence which runs: "The adaptive innovation of ancestral primates was not the invasion of the arboreal habitat, but their successful restriction to it".

The brachiating theme is pursued in the chapters by Lewis, by Roberts on the structure and function of the scapula, and by Tuttle and Basmajian. O. J. Lewis states his views once again on hominoid wrist morphology which lead him to espouse Sir Arthur Keith's theory of the brachiating origin of man in spite of the fact that Keith was basing his views on the gibbon, which Lewis clearly rejects from human ancestry on the grounds of wrist morphology.

Tuttle and Basmajian, reporting on an electromyographic study of forearm muscles of the gorilla, give considerable space to the well aired subject of knuckle-walking in African apes. Their contribution to the brachiating issue is, however, both moderate and sensible.

A valuable chapter on the mechanics of locomotion in primates by Badoux, an account of leaping in galagos by Jouffroy and Gasc and an interesting article on the much neglected field of postural activities in Old and New World monkeys by Michael Rose, complete the volume.

Primate Locomotion is an important source book for the graduate reader but the lack of overview and synthesis makes it an unsuitable text for the student.

J. R. Napier

. . . and adapting to other situations

The St Kitts Vervet. By Michael McGuire. (Contributions to Primatology. Vol. 1). Pp. xi + 202. (S. Karger: Basel, London and New York, 1974.) Sfr 56; £8.10; \$17.40.

VERVET monkeys were introduced to St Kitts, probably from West Africa, in the latter part of the seventeenth century. Today, they are common both in scrub-covered coastal areas and in the forested ravines running up into the island's central massif, and population densities are similar to those observed in several African field studies. McGuire's monograph reports the results of 6,300 hours of observation by nine observers in nine study areas, and covers population demography, activity patterns, social organisation and communication. Two main findings emerge. First, groups living in the ravines differ in dispersion, activity patterns, and in the frequency of grooming and agonistic interactions from those living in the coastal scrub. Second, 19% of the (54) communicative gestures observed in East African vervets by Struhsaker and 44% of the (48) vocalisations were not recorded in the St Kitts population.

Both comparisons make an important contribution to our knowledge of the adaptability of primate behaviour. Unfortunately, they are spoiled by inadequate quantification. In the first, the reader is asked to take on trust many of the suggested differences and no quantitative evidence is either shown or referred to. Where this is not the case, description of sampling methodology and sample size is scanty and no attempt is made to test the significance of the observed differences. In several cases, the measures used are inappropriate: for example, the "general tension state" of each group was measured by estimating its flight distance from an observer. Comparison with Struhsaker's study is largely confined to qualitative differences and is free from these objections. But the reader is required to accept the author's opinion that the absence of the communication patterns observed by Struhsaker is not the product of differences in classification rather than in behaviour.

A second major criticism of the monograph is the amount of turgid and unnecessary discussion which it contains. This suggests that, despite the impressive list of series editors on the front cover, little editorial attention was paid to the manuscript. As a result, material which would fill two medium length papers has expanded into a 200 page book.

T. H. Clutton-Brock

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ONCE upon a time young Englishmen would make 'the grand tour' through the countries of Europe as finishing touches to their educations. This book is just such a tour through selected areas of biochemistry. Despite a cautiously worded preface, it is unmistakably an audacious attempt to present, in one volume, the greater part of the background reading for advanced students of biochemistry.

There are 21 contributions by 25 different authors, most of them well known in the field of work they present. Of course, certain topics are inevitably sacrificed, and the emphasis is on structural aspects of biochemistry rather than on the traditional metabolic approach. In fact, the book has that unmistakable, if undefinable, molecular biology flavour. Certain topics which may seem to be of diminished interest to the main stream biochemist, such as B. A. Newton's article on "Protozoa as tools for the biochemist", compensate by conveying the enthusiasm of the contributor, and make surprisingly compulsive reading. Although some chapters sacrifice a certain degree of readability to information content, or *vice versa*, this is in no instance well marked, and all the contributions are presented in the style of a well written textbook.

The book falls naturally into two halves. The first deals with the biogenesis, structure, and function of biopolymers. Proteins, nucleic acids, polysaccharides, viruses, and bacterial and plant cell walls are discussed. Three contributions concern the application to proteins of chemical modification,

nuclear magnetic resonance, and electronic spectra and optical activity. This experimental emphasis on proteins makes it all the more surprising that no chapter has been dedicated completely to protein structure and stability. After all, equally important and complex discussions are presented for the secondary and tertiary structures of transfer RNAs and polysaccharides,

Biochemical grand tour

Barry Robson

Companion to Biochemistry: Selected Topics for Further Study. Edited by A. T. Bull, J. R. Lagnado, J. O. Thomas and K. F. Tipton. Pp. 700. (Longmans: London, June 1974.) £7.00.

systems which are in some respects less well understood. Two excellent articles together present a particularly comprehensive review of enzyme kinetics. The first of these introduces fast reaction techniques, emphasising their importance with the observation that the slowest step in an enzyme reaction typically has a half-life of seven milliseconds or less. The second presents material which will be more familiar to the average student, but even here the Michaelis-Menten equation and Lineweaver-Burk plot are infused with new life in such a way as to dispel the notion that the study of enzyme kinetics is "... an esoteric pursuit which

has little relevance to the general study of biochemistry".

The second half of the book is largely concerned with cells and their components. The emphasis is still structural, but several contributions give detailed consideration to the use of intact and living cells as tools for the biochemist. One of these introduces to the student the concept of the chemostat as a device for quantitative investigation of the growth kinetics of cell populations, and skillfully avoids incensing him against yet another "esoteric pursuit". An article which discusses the biochemistry of microbial pathogenicity is dropped right in the middle of these contributions, reminding the reader that micro-organisms are not simply nature's beneficial gift of a research tool.

The book concludes with a heterogeneous mixture of exceptionally good reviews on lysosomes and peroxisomes, mitochondrial oxidative phosphorylation, hormonal control, immunoglobulins and the contractile apparatus of muscle.

The preface states that the editors have tried to select topics that are poorly treated in the textbooks, or poorly understood by the average final year undergraduate. Despite the fact that many teachers will inevitably feel that neglect of their pet subject has not been justified by at least one of these conditions, the student will probably finish the epic journey from pages 1 to 700 with a sense of completeness and the feeling that this particular grand tour is a considerable *tour de force*.

Living with cosmic rays

Space Radiation Biology and Related Topics. By Tobias and Todd. Pp. xvi + 648. (Academic, subsidiary of Harcourt Brace Jovanovich: New York and London, May 1974.) £15.85.

It is perhaps surprising to see the publication of this volume now when support for space research is declining. It is, however, soon apparent that the manuscripts for all but the final chapter were conceived and written in the late 1960s when the achievements of the US space programme were at a climax and the future for space research looked a good deal brighter than it does today.

The 12 chapters of the book have been written by 16 authors, including the editors, who are expert in a wide range of subjects relating to space radiation biology. After a historical survey, two chapters are concerned

with the physics of radiations in space. In the first of these the composition and intensity of the radiation fields encountered around the Earth and in the Solar System are described in detail and the radiation doses received by astronauts taking part in the US missions up to Apollo 11, the first lunar landing, are tabulated. In the second, consideration is given to techniques for the simulation of the heavy-ion component of space radiation fields using accelerators.

From chapter 4 onwards the emphasis is on biology, commencing with a review of cellular radiation biology with special reference to heavy-ion radiations. This is followed by a review of the various theories of molecular and biological evolution, again with special reference to the possible role of solar and galactic radiation. One chapter is concerned with the effects of magnetic field on biological systems and another with the results of experiments carried out in satellites where weightlessness can modify the effects of radiation. The

three following chapters deal with mammalian radiobiology in general, the influence on the biological response to radiation of circadian rhythm and the acute and late effects of radiation observed in man.

Chapter 11 is a discussion of the mathematical models used to described recovery from radiation induced injury and the effects of radiation on ageing. The final chapter entitled 'Current Topics in Space Radiation Biology' briefly refers to some of the more important developments that have taken place up to 1972.

The volume is very readable, well referenced and adequately indexed and will no doubt be read with interest by both biologists and physicists working in areas related to space radiation biology. It is, then, a great pity that the reader is so often reminded by, for example, the absence of any mention of the chemistry of interstellar clouds in the chapter on evolution, that the book was written several years ago.

K. F. Baverstock

North American fish fauna

Freshwater Fishes of Canada. By W. B. Scott and E. J. Crossman. Pp. xi+966. (Fisheries Research Board of Canada Bulletin No. 184.) (Information Canada, Ottawa, 1973.) \$9.75.

THE Fisheries Research Board of Canada has a most creditable publishing record on both fish and fisheries. By commissioning a series of excellent publications on the fishes of the major geographical regions of Canada, published in its Bulletin series, it has made the Canadian fish fauna the best documented in the world. In this, the latest of the series, the authors W. B. Scott and E. J. Crossman faced a formidable task in critically surveying the fishes of northern North America, a fauna which in part is poorly studied biologically and is beset with taxonomic problems, while the remainder (mostly the economically valuable species) has been studied in depth. The authors' achievement in bringing together the information in a large literature (over 1,400 references are cited) is alone a major contribution, and their original work welds the whole together to form one of the best books on a major fish fauna to be published this century.

The plan of the book is essentially a systematic account which commences with a key to the families of freshwater fishes in Canada. This relies on simple characters and is amply illustrated; it is clearly a key designed for use by others than systematic ichthyologists—and is all the better for that. Each family is preceded by a key to the species included in the fauna, and though these are necessarily more technical in nature they are still practical keys. The practical nature of the

means of identification is well illustrated by a page of line drawings devoted to the parr of the species of *Oncorhynchus*, *Salmo*, and *Salvelinus*—confusing the young stages of salmon, trout, and charr.

Each account is illustrated by a drawing of the fish and has text under headings such as description, colour, systematic notes, distribution, biology, relation to man, nomenclature, etymology and common names. Of these headings, biology is frequently the most detailed, especially in the case of economically important species, and provides data on spawning, growth, age at sexual maturity, habitat, food competitors and predators, and parasites.

The more general parts of the book are confined to a brief note on Canada and its fish fauna, which is essentially a listing of the 181 species by geographical area and drainage basin. This seems to be a rather terse treatment of the zoogeography of Canada's fishes strangely so in contrast with the detailed zoogeographic discussions in McPhail and Lindsey's *Freshwater Fishes of north-western Canada and Alaska* (1970).

Such criticisms as one needs to make of Scott and Crossman's masterly treatment of the Canadian fish fauna are in extra-limital areas. For example, although most books on European fishes report the occurrence of *Ictalurus nebulosus* as introduced to Europe, the only specimens critically examined have proved to be *I. melas*. Surely too, it is time to forget about the death of Henry I of England in 1135 which is said (page 74) to have been caused by eating lampreys (*Petromyzon marinus*)—although the lampren (*Lampetra fluviatilis*) was more likely to have been involved.

Alwyne Wheeler

Nice balance for ecologists

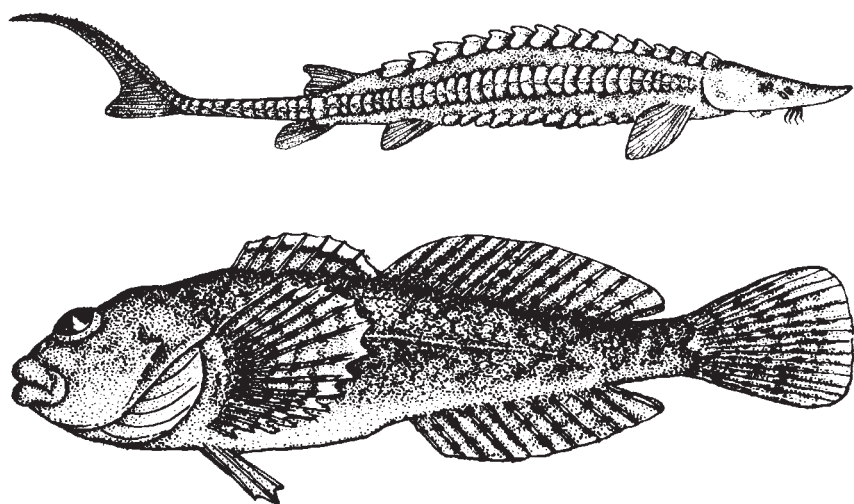
Ecology, with Special Reference to Animals and Man. By Charles Kendeigh. Pp. vi+474. (Prentice-Hall: Englewood Cliffs, New Jersey, 1974.) \$16.95.

ONE of the most frequent criticisms levelled at the examination scripts of modern ecology students is that their knowledge of ecological principles is not accompanied by evidence of experience of living organisms in the field. Ecological field courses were once exercises in field identification; now the recognition of plants and animals has become subservient to studies of ecosystem organisation, trophic structures, population dynamics, pattern analyses, and so on. The pendulum seems to have swung to the other extreme.

Charles Kendeigh's book is unusual in that it attempts to maintain a balance between theoretical principles and detailed accounts of field studies. He achieves this by the insertion of a section which considers certain habitats in detail, including both aquatic and terrestrial examples. By placing this section early in the book, immediately following an introductory section, he demonstrates his conviction that the understanding of ecological principles can only be achieved once the student has become familiar with the diversity of organisms and the complexity of the environment which is to be found in almost any habitat subjected to critical observation. I find this approach both stimulating and refreshing.

There follow three theoretical sections, dealing with ecosystem ecology (nutrient cycling, energy flow, food webs), population ecology and evolutionary ecology. Attempts to simplify language and concept in these sections have occasionally led to misleading expressions, such as the 'energy cycle' and the 'impervious' nature of carbon dioxide and water vapour to long wavelength radiation. Coverage is fairly thorough, however, especially in the section on energy flow, and the relevance of these concepts to the human manipulation and exploitation of nature is stressed. The concept of succession is mentioned on several occasions, but a detailed theoretical consideration of this important aspect of ecosystem ecology is lacking.

The coverage of biomes and geographical ecology lacks much of the flow of previous sections. It comprises largely a series of lists of birds and mammals for North American examples of the major biomes. One pleasing feature of these chapters is the inclusion of a few paragraphs dealing with the palaeoecology of the American



The shovelnose sturgeon, *Scaphirhynchus platyrhynchus*, (above), and the slimy sculpin, *Cottus cognatus*. From *Northern Fishes*; third edition. By Samuel Eddy and James C. Underhill. Pp. xix+414. (University of Minnesota: Minneapolis: Oxford University: London, October 1974.) £10.00.

biomes, particularly during the Pleistocene.

The final chapter, on marine ecology, is rather sketchy and superficial. It gives the impression of being an afterthought.

In my opinion there are three major weaknesses in this book when it is considered as a possible undergraduate text. The first is its lack of information concerning plants. This is particularly noticeable and unfortunate in the chapters on habitat, where it would have completed an otherwise detailed and interesting series of accounts. Although this omission is confessed frankly in the sub-title, it nevertheless reduces the usefulness of the book as an ecological teaching tool. The second weakness from the point of view of British ecologists is its almost exclusive use of North American examples. This must limit severely its value and its sales potential outside the United States and Canada. The third way in which the book could have been improved is by the inclusion of more physiological information about the animals considered. I cannot agree with the author's statement in the Preface that such information is only of value at advanced levels of training.

Despite these reservations I found the style, content and production of this book attractive and interesting.

Peter D. Moore

What is biological control?

Biological Control of Plant Pathogens. By K. F. Baker, and R. J. Cook. Pp. xiv+433. (Freeman: San Francisco, 1974.) \$12.50.

THE authors of this book admit that writing it was a "mind-stretching" exercise; many readers may think that the effort has somewhat distended their subject. The dilemma faced by the authors was how far to extend beyond the difficulty of defining 'biological control' into the wider problems of managing microbial ecology to lessen plant diseases. Early usage of the term fitted well into S. D. Garrett's statement (in *Pathogenic root-infecting fungi*, Cambridge University Press 1970) that biological control is "mediated by one or more organisms (excepting man himself) outside the host-parasite relationship". On page 43 of this book the authors arrive at their much broader and wordier concept: "Biological control is the reduction of inoculum density or disease-producing activities of a pathogen or parasite in its active or dormant state by one or more organisms, accomplished naturally or through manipulation of the environment, host or

antagonist, or by mass introduction of one or more antagonists."

This concept is so broad that it embraces all of microbial ecology and much of agriculture, except the use of chemicals aimed solely against pathogens. It is of course commendable that plant pathologists should consider all the factors affecting the biology of plant pathogens but, despite the inevitable demarcation disputes, many would share my preference to restrict the term 'biological control' to the manipulation of 'third organisms' such as hyperparasites, antagonists and competitors. A more comprehensive term would be needed to comprise the important natural environmental and artificial factors which are



The hands of a workman who had been operating a pneumatic drill for 26 years. The prolonged vibration caused stiffness swelling and, finally, dry gangrene in the fingers. From *The Vibration Syndrome*. Edited by W. Taylor. Pp. xii+226. (Academic Press: London, August 1974.) £6.00; \$15.50.

included as biological in this book; for example, tillage, choice of planting date, and organic and inorganic additives (including fertilisers). In Chapter 9 the authors come perilously close to accepting chemotherapeutants as another factor, thus removing the chief objection to adopting the well established term 'integrated control'.

The first two chapters contain a somewhat unhappy blend of unnecessarily elementary introduction, good sense, and an emotional attitude to unbalanced 'environmentalist' arguments. Both authors have, however, enviable reputations as root pathologists and most of the book comprises a skilled and up to date compilation of knowledge concerning root pathogens. It is comforting to see one chapter devoted to pathogens of aerial parts; but that occupies a mere 20 of the 350 pages of text and so can be no more than an apology to satisfy the completeness that the title suggests. In their preface the authors disclaim comprehensiveness and admit that they have often put a personal interpretation on results which is different from that of the authors they quote. That this is a risky procedure is reinforced

by several authors who claim to have been misrepresented, which breeds doubts about inaccuracies elsewhere.

Inevitably, much of the evidence from the literature is anecdotal and biased, because successes are more often reported than failures (which may be more frequent). With a few notable exceptions it is therefore very difficult to obtain balanced estimates of the value to agriculture of modifications to microbial ecology or control by 'third organisms'. Experience with the decline phenomenon in take-all disease of cereals (*Gaumannomyces graminis*) suggests that the variability between cropping histories, soils and fields is so great that it will be long before we can confidently predict the effects of antagonists or of developments in biological buffering. These are not, however, reasons for criticising a book that collects together much scattered information, presents it systematically for the first time, and contains more than the usual amount of constructive thinking.

Plainly, the authors did not want this to be an ordinary book; it is not. It will periodically delight, interest and infuriate its readers; but these seem to be characteristics of the books that prove most formative to their subjects and I think time will show this book to be important.

J. M. Hirst

Of mites and models

The Functional Response to Prey Density in an Acarine System. (Simulation Monographs.) By H. G. Fransz. Pp. viii+143. (Centre for Agricultural Publishing and Documentation: Wageningen, 1974.) Dfl.20.

DURING the last few years ecological theory seems to have outpaced the collection of ecological data. Professor Maynard Smith's book *Models in Ecology* (Cambridge University Press, 1974) showed amply that models can be formulated and built, but that their testing on real data and validation by prediction in the real world is still not possible because of a shortage of data. It was with these thoughts in mind that I welcomed Fransz's book which describes systems whereby the mite *Typhlodromus occidentalis* preys on various stages of another mite *Tetranychus urticae*. The book combines the observational approach to this predator-prey system with model building and validation by comparison with experimental results.

The first two chapters provide an introduction to the experimental system and the variables that the author measured (prey density, hunger, locomotion velocity and so on), and the third chapter describes the results of

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experiments that measured these variables. Multiple regression analysis, apparently without significance tests, is frequently used to relate the different variables. The fourth chapter is concerned with building models, and the final chapter compares the output of models with the experimental data, demonstrating that stochastic models generally give a better fit to data than deterministic models.

The book does achieve the objective of building and testing models, but its style closely resembles the thesis that it is. The use of Fortran-type variables throughout the text and in tables and figures makes the book difficult to read, necessitating continual reference to Appendix IV where one needs to look up the root variable name as well as prefixes and suffixes. The information in the book is diffuse and space is wasted with unnecessary simple computer programmes and procedures. The essential points of model building and of testing on experimental data could have been written much more concisely, and probably with more effect, as an article in a journal.

M. B. Usher

Drugs and toxins

The Chemistry, Metabolism of Drugs and Toxins: An introduction to Xenobiochemistry. By Michael and Maxine Briggs. Pp. xii+386. (Heinemann Medical: London, June 1974.) £5.00.

THIS *pot-pourri* of data from chemistry, biochemistry, biochemical pharmacology, pharmacology and toxicology is put together in four chapters entitled, respectively, Metabolism of foreign compounds, Biochemistry of drugs, Natural antimetabolites, and Venoms. It is given the sub-title "Xenobiochemistry" which is not new (see *Nature*, 187, 94; 1960). The first chapter contains a somewhat superficial account of drug metabolism and the second tries to cover the types, absorption, transport, elimination, mode of action and side effects of drugs, but has 50 pages of tables with several half-empty pages. The third chapter covers microbial toxins, antibodies and antimetabolites whereas the fourth is on the composition and action of all kinds of animal and plant venoms. The book has attempted to concentrate too much into too little and this results in some wrong impressions, as in the case of thalidomide (p. 192) which is known to undergo spontaneous hydrolysis *in vivo* and *in vitro* rather than the enzymic metabolism implied by the book. The chapters on antimetabolites and venoms are, however, useful introductions to the study of these toxins.

R. T. Williams

(Continued from page 128)

eluted the agglutinin with D-galactose (ref. 22 and details to be published). Based on specific agglutination titre a 75-fold purification with 50% recovery was achieved. Since the product was pure by electrophoretic criteria (Fig. 2) this result indicates that the agglutinin constitutes approximately 1% of the total extractable protein in cohesive *P. pallidum* cells. The subunit molecular weight of the *P. pallidum* protein is estimated to be about 25,000 (Fig. 2) as compared with 26,000 for discoidin¹¹; its isoelectric point is 7.0 (details to be published) whereas the value is 6.1 for discoidin¹¹. The two agglutinins also differed in their interaction with a wide range of sugars (Table 1). For discoidin, the most potent inhibitors were N-acetyl-D-galactosamine and 3-O-methyl D-glucose. In contrast, the *P. pallidum* agglutinin required 15 times as much of the former and 60 times as much of the latter for comparable inhibition but was eight times more sensitive to lactose and four times more sensitive to D-galactose.

Since the protein agglutinated erythrocytes we determined if it also agglutinated *P. pallidum* cells. To test this we added the pure protein to cohesive *P. pallidum* cells which had been

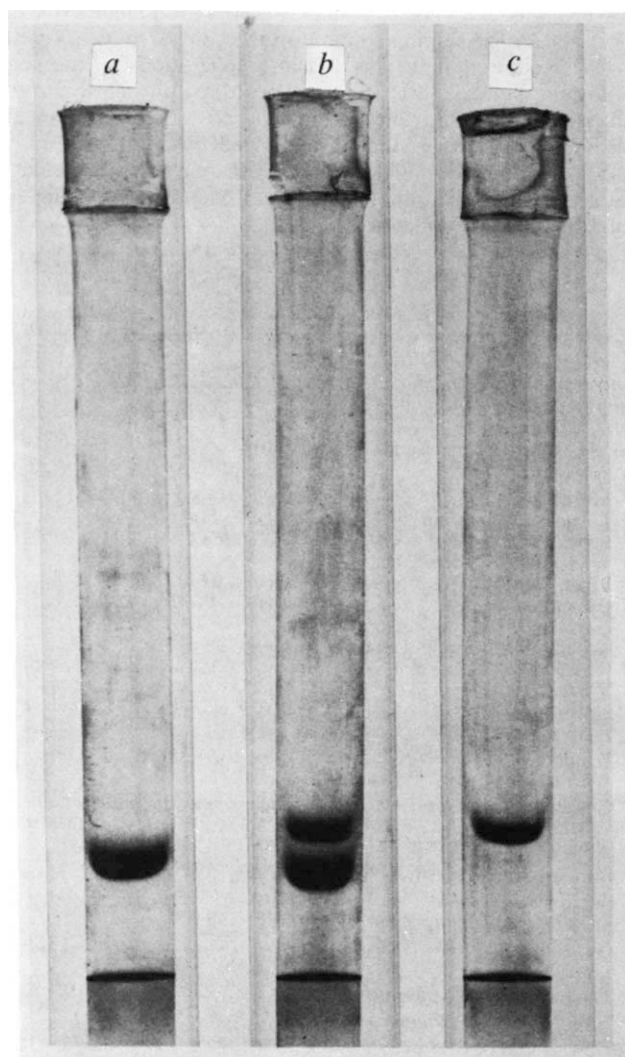


Fig. 2 Polyacrylamide gel electrophoresis of purified agglutinins from *P. pallidum* and *D. discoideum*. Approximately 25 μ g of each protein was applied to a discontinuous sodium dodecyl sulphate (SDS)-polyacrylamide system²³ with a 3% acrylamide stacking gel and an 8.0% separating gel as described previously¹¹. To assure complete solubilisation, samples were heated at 100°C for 5 min immediately before application to gels. Cytochrome c (5 μ g) was added routinely to samples as a reference protein. The discoidin preparation and molecular weight calibration were as described previously¹¹. a, *P. pallidum*; b, mixture; c, *D. discoideum*.

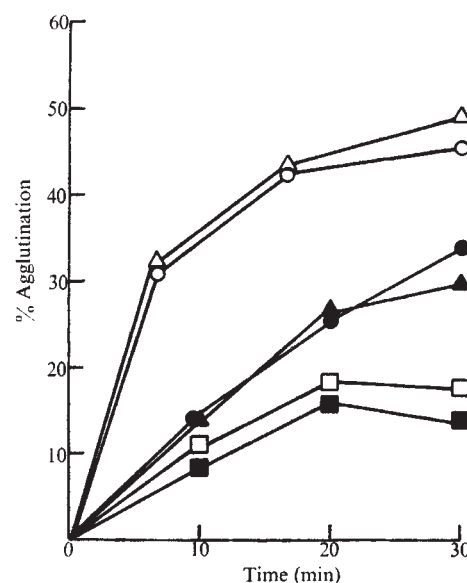


Fig. 3 Agglutination of heat-treated (60°C) *P. pallidum* cells by purified agglutinin. Cells were collected from 90-h plates, washed and suspended, 10^7 ml⁻¹, in 16.7 mM Na₂HPO₄-KH₂PO₄ (pH 6.0) buffer, and heated at 60°C for 10 min. Agglutinin was purified and then extensively dialysed against PBS. Since there was partial loss of activity, the concentration of active agglutinin was estimated on the basis of agglutination activity. Agglutination assays were done in plastic trays with 16 mm wells (Linbro FB-54). Each well held a total of 0.5 ml consisting of 2×10^8 cells ml⁻¹ plus one of the following sets of constituents: (▲) PBS; (△) purified agglutinin (0.5 μ g ml⁻¹); (□) purified agglutinin (0.5 μ g ml⁻¹)+D-galactose (0.2 M); (●) purified agglutinin (0.5 μ g ml⁻¹)+D-glucose (0.2 M); (■) 0.2 M galactose; (●) 0.2 M D-glucose. A separate well was used for each time point. The tray was gyrated at 115 r.p.m. on a New Brunswick G-24 shaker (23°C). At 10 min intervals the contents of wells were carefully removed, diluted in 20 ml of saline and single cells were counted with a Coulter counter (100 μ m aperture; 1/aperture current=1/4; 1/amplitude=2; threshold=20-80). The percentage agglutination was expressed as the percentage of single cells that had disappeared relative to time 0 (ref. 24).

heated at 60°C for 10 min to reduce their endogenous agglutination (Fig. 3). Agglutination was assayed by measuring the disappearance of single cells in a gyrated suspension^{24,25}. The purified protein (0.5 μ g ml⁻¹) agglutinated the *P. pallidum* cells; and this agglutination was blocked by 0.2 M D-galactose, but not by 0.2 M D-glucose (Fig. 3).

We next looked for agglutinin on the surface of cohesive cells. When erythrocytes and cohesive *P. pallidum* cells were mixed and shaken on a slide, large mixed clumps formed within a minute, consisting of slime mould cells in contact with one another and with erythrocytes. D-Galactose (0.15 M) blocked formation of these clumps whereas D-glucose did not. To test the possibility that erythrocytes were being agglutinated because of secretion or release of protein from the slime mould cells, we maintained cells in medium for 10 min, then spun them out, and tested the supernatant for erythrocyte agglutination activity. Since no agglutination was observed, this suggested that the agglutinin is not secreted and is therefore present on the surface of the slime mould cells.

In view of this finding we investigated whether the saccharides that interact with this protein blocked the endogenous cohesiveness of the slime mould cells. In a previous experiment (Fig. 3) we found that cells heated to 60°C for 10 min retained some cohesiveness. With a milder heat treatment (51°C for 10 min) the cells were significantly more cohesive. (It should be noted that purified factor retained about 50% of its erythrocyte agglutination activity when heated under these conditions.) Determining agglutination by measuring the disappearance of single cells, we examined the effects of several sugars on this residual cohesiveness. D-Galactose and lactose were effective

inhibitors down to 6 mM whereas D-glucose and D-mannose were effective only at 500 mM (Fig. 4a). When living, untreated *P. pallidum* cells were used, differential sugar inhibition of cohesiveness was again observed (Fig. 4b and c); but compared with the results with heat-treated cells, the concentrations of sugars required for differential inhibition were about 15 times higher. The difference between heat treated and living cells may be due to (a) more functional agglutinin in living cells; (b) higher binding affinity between agglutinin and its receptor in living cells; or (c) secondary adjunct binding sites in living cells.

Our findings support a role for the carbohydrate-binding protein in intercellular adhesion in *P. pallidum*, although its participation in other differentiative processes should be

considered. Our working hypothesis is that this protein is a cell-ligand²⁶ that binds slime mould amoebae together by attaching to carbohydrate-containing receptors on adjacent cells. The basis of the erythrocyte agglutination assay would derive from a structural resemblance between the erythrocyte surface oligosaccharides and the native slime mould receptor. Thus, under this model, there would be two principal components in the adhesion system: a multivalent carbohydrate-binding protein which is a peripheral membrane protein²⁷ and a carbohydrate receptor possibly associated with an integral membrane component²⁷. Carbohydrate-containing macromolecules have been implicated in cell adhesion in several other systems, including microbe-host cell interactions¹⁸⁻³⁰, mating reactions of bacteria³¹, yeast³² and *Chlamydomonas*³³ and cell-cell interactions in tissue formation^{3,34-36}.

Finally, we suggest that the difference in the carbohydrate-binding specificities of the agglutinins from *D. discoideum* and *P. pallidum* may underlie the selective intercellular affinities exhibited by cells of these species³⁷⁻³⁹. Further work is required to test our model for slime moulds and to determine its relevance in the analysis of specific cellular interactions in higher systems.

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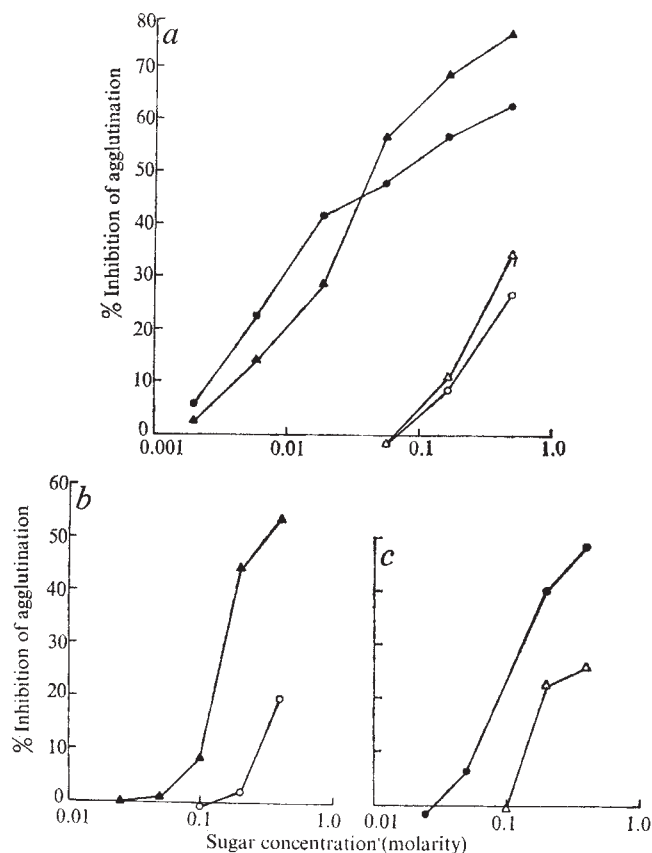


Fig. 4 Effect of sugars on agglutination of heat-treated (51°C) (a) or normal (b, and c) *P. pallidum* cells. a, Cells from 90 h plates were suspended at 10^7 ml⁻¹ in 16.7 mM Na₂HPO₄-KH₂PO₄ pH 6.0, buffer and heated at 51°C for 10 min. The cells were then washed and dispersed in the same buffer. Each well of the Linbro tray contained 2×10^6 cells ml⁻¹ plus PBS buffer containing the indicated concentration of sugar (0.5 ml total volume). There were three replicate wells for each treatment. The plate was gyrated at 115 r.p.m. for 60 min, by which time agglutination had reached a plateau. The contents of each well were diluted and counted as in Fig. 3 to determine the percentage reduction in agglutination relative to controls containing no sugar. The selective inhibition of agglutination seen quantitatively in the figure was verified qualitatively by visual inspection (b and c). Cells were collected from 93-96 h plates. The cells were suspended and dispersed into EDTA-phosphate buffer (Fig. 1 legend). Each well of the Linbro tray contained 3×10^6 cells ml⁻¹ plus EDTA-phosphate buffer or sugar in EDTA-phosphate buffer at different concentration (0.5 ml total volume). Galactose (▲) and mannose (○) were compared in one experiment (b) and lactose (●) and glucose (△) in the other (c). There were three replicate wells for each treatment. The tray was gyrated at 115 r.p.m. for 60 min, a period sufficient to attain equilibrium. The contents of each well were diluted into 20 ml of EDTA-phosphate buffer and single cells were counted (100 μ m aperture; 1/aperture current=0.354, 1 A=1, thresholds=10-60). As above, the percentage reduction in agglutination was determined for each sugar. The selective sugar effects were verified visually.

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Cyclic expression of a growth conditioning factor (MGF) on the cell surface

THE cell surface has been implicated as a regulation site for cell division in various systems. Enzymatic alteration of the cell surface can initiate DNA synthesis in contact inhibited cells¹⁻³ and changes in membrane components have been correlated with the expression of the transformed phenotype⁴⁻¹⁰ and with the entry into the S phase of normal cells¹¹. We describe here a system in which the induction into DNA synthesis of a non-dividing cell population is regulated by a factor exteriorised only during the S and mitosis periods at the surface of the producer cells.

Peritoneal macrophages can be maintained *in vitro* but do not proliferate; they can, however, be induced to synthesise DNA and divide in medium conditioned by cells of the same species¹²⁻¹⁴. In our system medium conditioned by L cells in logarithmic growth was used and tested on macrophages collected from the peritoneal cavities of starch-inoculated C57BL/6J mice as described previously¹⁵. The assays were performed in Lab-Tek four chamber slides seeded with 3.0×10^5 nucleated peritoneal cells per chamber. Thirty minutes after seeding, the cultures were washed twice with phosphate buffered saline (PBS) to remove unattached cells, and refed. After 2-3 d the desired concentration of samples was diluted in TES-HEPES-buffered Eagle's medium, pH 7.6 (ref. 15), with 10% foetal calf serum (FCS) and added to macrophages in the presence of $0.2 \mu\text{Ci ml}^{-1}$ ³H-thymidine (dT) (specific activity, 52 Ci mmol⁻¹). After 5 d the cells were fixed with Carnoy's solution and prepared for autoradiography.

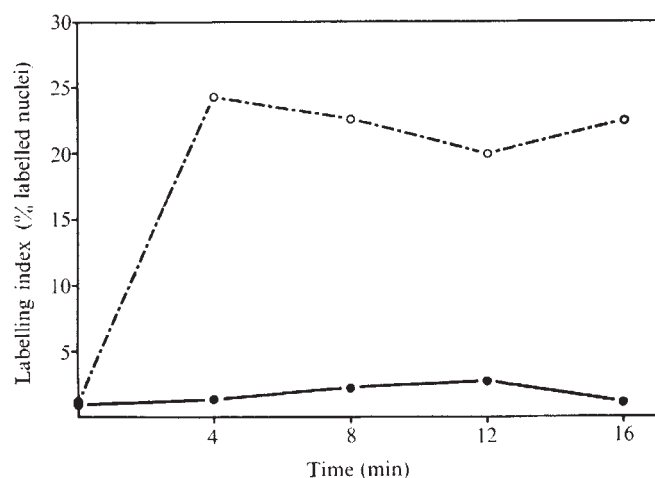


Fig. 1 Release of MGF with increasing time of trypsin treatment. Incubation of 2 ml of 0.1% trypsin (○---○) or medium alone (●—●) with duplicate milk dilution bottles of L cells previously washed twice with PBS. Reaction stopped with 10% FCS. All samples assayed in duplicate at a trypsin digest concentration of 40%. Assay was for 5 d with a continuous label of $0.2 \mu\text{Ci ml}^{-1}$ dT.

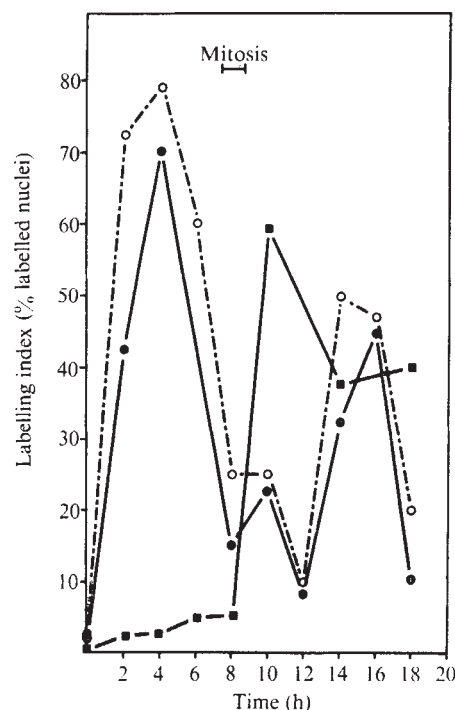


Fig. 2 Spontaneous and tryptic release of MGF from synchronised L cells. L cells seeded 24 h previously, 1×10^6 cells per 60 mm Petri dish were synchronised with 10^{-8} M dT as described in the text. At different times after release, L cell coverslips were pulsed for 20 min with $2.0 \mu\text{Ci ml}^{-1}$ dT (specific activity 52 Ci mmol⁻¹). Coverslips were fixed in Carnoy's solution for autoradiography. Cells were read for labelled nuclei (○---○). At the times indicated duplicate L cell cultures were trypsinised with 0.1% trypsin for 5 min and the reaction was stopped with 10% FCS. Samples of medium incubated with the cells from the time of release from the dT block were taken. Trypsin digests (●—●) and conditioned medium (■—■) were assayed on macrophages at a concentration of 40% for 5 d with $0.2 \mu\text{Ci ml}^{-1}$ dT. Autoradiography was performed as described previously.

The presence of macrophage growth factor (MGF) not only in the conditioned medium, but also in cell lysates was demonstrated as follows. Suspensions of 4×10^6 L cells per ml were washed repeatedly and sonicated for 3 min at 30-s intervals. Dilutions of whole sonicates were assayed on macrophages for stimulatory activity. An increase in the concentration of crude cell lysates increased the number of labelled macrophages. To determine whether stimulation was specific, two mouse cell lines which do not produce active conditioned medium were sonicated and tested for activity: neither showed stimulatory activity in their lysates (data not shown).

To determine the cellular localisation of the MGF, L cells were treated with 0.1% trypsin (Flow Laboratories) 24 h after plating at 37° C for periods of 0-16 min; trypsinisation was terminated with 10% FCS or soybean trypsin inhibitor. Samples were filtered through 0.22 μm filters to eliminate contaminating L cells. As Fig. 1 shows, maximal activity was released at 4 min; 0.05% purified trypsin (Worthington) released comparable amounts of activity (data not shown). No detectable MGF was released when cells were incubated with medium alone during the experimental period.

Since changes occur in the components of the cell surface during the cell cycle we investigated whether MGF was released by trypsin only during certain phases of the cycle. L cells were synchronised by a double dT block (10^{-8} M dT for 16 h; 6 h release, followed by another 16 h block). At 2 h after release, 70% of the cells were in the S phase as shown by autoradiography after a 20-s pulse with dT (Fig. 2). Mitosis (30%) followed 8 h after release; a second round of DNA synthesis followed 12 h after the first.

At different times in the cell cycle samples of medium were taken to test for spontaneously released MGF, and L cells were

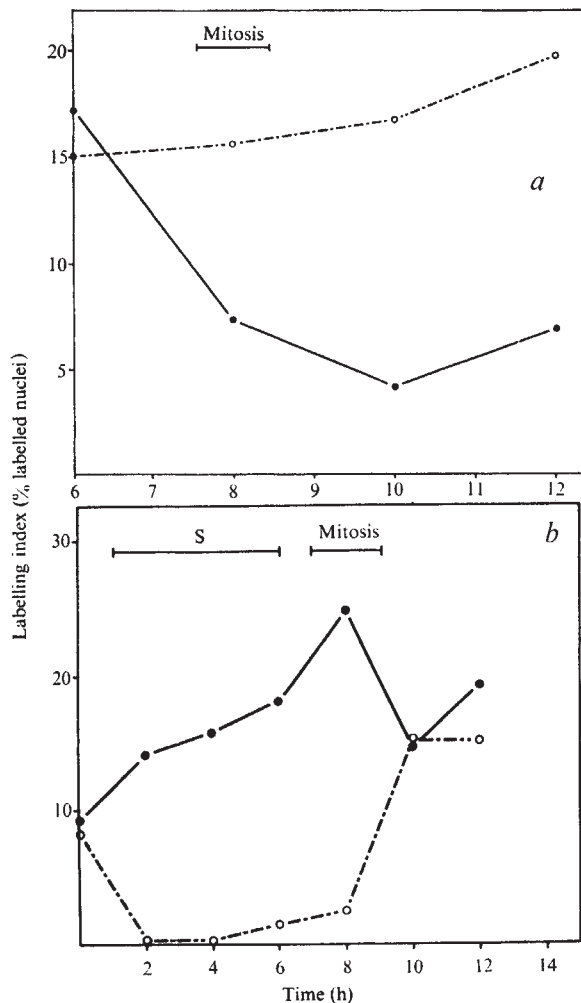


Fig. 3 *a*, Trypsin release of MGF in cells blocked during mitosis. L cells synchronised with a double dT block were released (time 0) and 5 h later blocked with $0.03 \mu\text{g ml}^{-1}$ vinblastine sulphate (Velban, Lilly) (○---○) or refed with medium alone (●—●). At the times shown, 5 min duplicate trypsin digests were taken. Samples were assayed at a concentration of 40% under standard conditions. *b*, MGF in lysates from synchronised L cells. L cells synchronised by a double dT block as described in the text were scraped from two 60-mm Petri dishes into 2 ml of TES-HEPES-buffered Eagle's medium (pH 7.6) with 10% FCS and sonicated for 30-s intervals totalling 3 min. ●—●, Points were taken every 2 h from time of release from dT. At the same times, sister cultures were trypsinised for 5 min (this did not remove cells from the glass). These cells were then treated similarly for sonication. ○---○, All sonicates were assayed at a concentration of 10% for 5 d in the continuous presence of $0.2 \mu\text{Ci ml}^{-1}$ dT. Slides were prepared for autoradiography as described previously.

trypsinised for 5 min with 0.1% trypsin at 37°C . Under these conditions, there was no alteration in cell morphology and viability remained at 95%. Figure 2 shows that MGF activity could be trypsin-released from cells only during the S phase, with almost no activity recoverable when DNA synthesis stopped; activity reappeared again at the next S phase. The spontaneously released MGF appeared in the medium as the activity disappeared from the surface.

It was not clear in these experiments whether MGF was released before or after mitosis. To resolve this, L cells were synchronised with a double dT block, then released, and 5 h later, in the middle of S phase, arrested with 0.03 g ml^{-1} of vinblastine sulphate. In the control cells, as expected, no MGF was released by trypsin during G_1 ; in the cells blocked in mitosis instead, MGF could be released as late as 12 h after removal of the dT block (Fig. 3a). It seemed therefore that MGF remained associated with the cell surface in cells blocked at mitosis.

The above results indicated cell cycle dependency for the presence of MGF in the trypsin-releasable material both at the cell surface and in the medium. To understand better the dynamics of the distribution of MGF during the cycle, localisation in the whole cell lysate was compared with that remaining in the cells after trypsin treatment. Parallel cultures were synchronised as before. MGF was assayed in the whole lysate from cultures previously treated with trypsin or untreated. All points were taken from duplicate assays of lysates from two 60-mm Petri dishes each containing 3×10^6 to 4×10^6 L cells. Figure 3b shows that MGF activity was present in cell lysates at all times in the cell cycle. Trypsinisation of the cells before sonication eliminated activity in lysates only during S and mitosis, confirming previous results with trypsin digests.

On the basis of the results of this study, the following interpretation can be made: (1) MGF is present and presumably synthesised at all times in the cell cycle; the buildup of activity seen before transfer to the surface and release supports this. (2) MGF is transferred to the surface during S phase, where it remains until after metaphase when it is released into the medium.

Previous work in our laboratory has shown that MGF present in L cell-conditioned medium was stable to various enzymes and treatments, but was destroyed by periodate and by subjection to 100°C for 10 min (ref. 12). These studies indicate that the active moiety contains carbohydrate. This agrees with evidence that trypsin releases glycopeptides from the surface of plasma membranes (for a review see ref. 22). The mode of synthesis and transfer of this factor as described here is in accord with the model of synthesis of glycoproteins proposed by Schachter and Roden¹⁶. These authors reported that glycoproteins are synthesised intracellularly on polyribosomes and as glycoproteins transported through the Golgi apparatus to the cell periphery¹⁶. Several growth regulatory factors have been isolated and identified as glycoproteins, attesting to the significance of this class of molecules in the control of cell proliferation¹⁷⁻²⁰.

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An electrophysiological study of the hippocampus of the 'reeler' mutant mouse

The laminar organisation of neurones in the brain of the homozygous 'reeler' mutant mouse¹ is grossly disrupted, especially in the cerebellum, hippocampus and neocortex²⁻⁶. Although a number of histological studies on synaptic organisation in the cerebellum of various mutants have been reported⁷⁻⁹, the effect of such disorganisation on interneuronal connections has not been investigated electrophysiologically, either in the cerebellum or elsewhere.

Here we report evidence which suggests that there is a high safety factor in the developmental processes responsible for ensuring orderly synaptic organisation. Despite the cellular disarray in the hippocampus of the homozygous reeler, the resultant synaptic organisation as revealed by electrophysiological techniques is essentially normal.

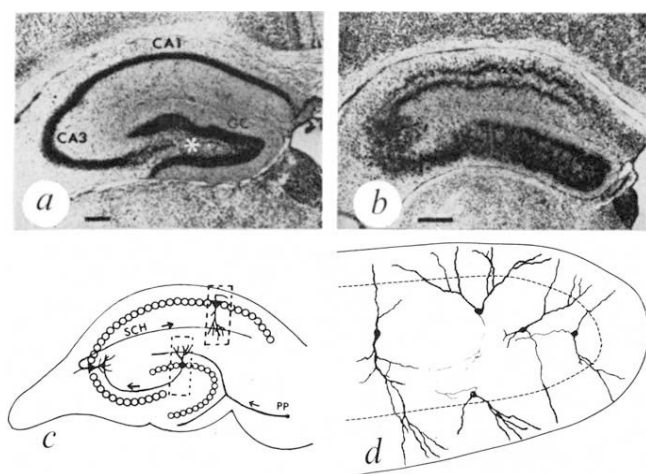


Fig. 1 Anatomy of normal and reeler hippocampus. Transverse sections through the hippocampal formation of 18-d-old normal (*a*) and 'reeler' (*b*) littermates. Cresyl fast violet stain. Asterisk indicates the hilus of the dentate area. CA1 and CA3, pyramidal cell fields CA1 and CA3 of Lorente de Nó (1934); GC, granule cells. Bars in *a* and *b* denote 200 μ m. *c*, Diagram of the main excitatory pathways in the hippocampal formation. Arrows give the direction of impulse traffic. Dashed rectangles represent approximate regions of the hippocampal formation from which the data of Figs 2 and 3 were obtained. PP: perforant path; SCH: Schaffer collaterals. *d*, Composite camera lucida drawing of granule cells in the dentate area of 'reeler' mice, impregnated by the rapid Golgi method. Dashed outline indicates the boundary of the region occupied by granule cell bodies. Bar denotes 100 μ m.

The extent of the defective alignment of cells in the hippocampus is shown in Fig. 1. In the normal animal both the pyramidal cells of regions CA3 and CA1 and the granule cells of the dentate area are arranged in tightly packed layers. In the reeler, the rigid layering of cells seen in the normal animal is absent; pyramidal cells are loosely packed, with occasional discontinuities and in region CA1 form two distinct layers, while granule cells are scattered widely throughout the hilar region of the dentate area.

The synaptic organisation of the normal hippocampus has been studied both histologically¹⁰ and electrophysiologically^{11, 12}. The axons of the perforant path (PP in Fig. 1*c*), the main input

to the hippocampal formation, form *en passant* synaptic contacts with the dendrites of granule cells. Pyramidal cells in region CA3 of the hippocampus, to which axons of the granule cells project, extend myelinated Schaffer collaterals (SCH in Fig. 1*c*) to the apical dendrites of pyramidal cells in region CA1.

We have investigated the synaptic organisation of the perforant path and Schaffer collateral systems in reeler using the technique of field potential analysis. Wild-type or heterozygous littermates were used as controls.

When the perforant path of the normal animal is electrically stimulated, the synchronous depolarisation of the granule cell dendrites results in an inward flow of membrane current in the synaptic region; this inward current is passively extruded from the unactivated regions of the cell population, principally from the cell body layer. With an extracellular electrode, the distribution of membrane current is reflected as a negative potential in the region of the active current sink, reversing to a positive potential as the electrode approaches the cell body layer. A curve conventionally referred to as the 'depth profile' of the response can be obtained by plotting the amplitude of the

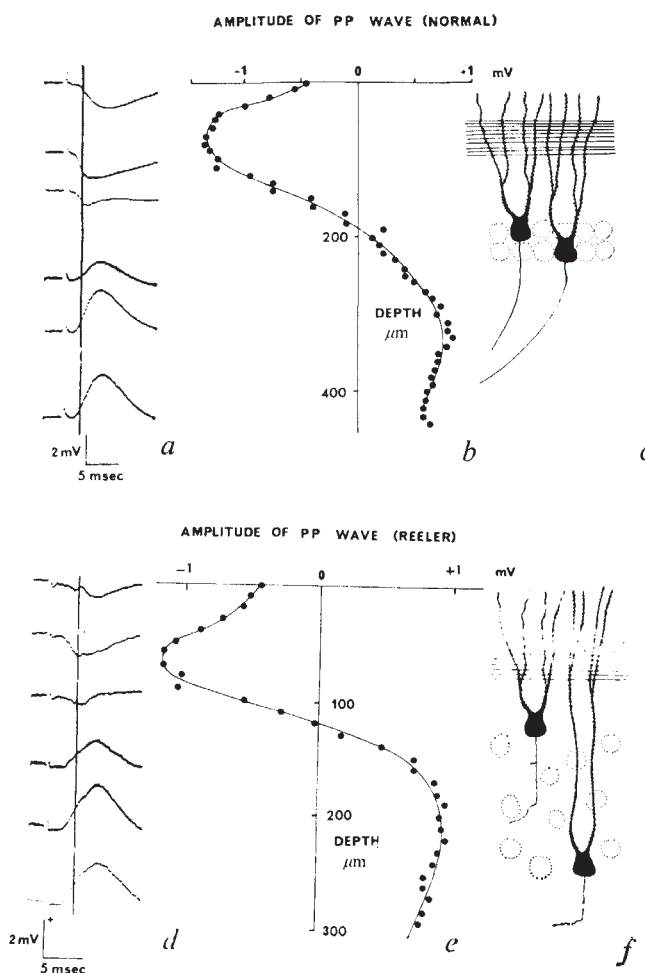


Fig. 2 Depth profiles of the perforant path (PP) wave in normal (*a-c*) and reeler (*d-f*) littermates. Samples of the synaptic waveforms are displayed on the left (*a, d*); the position of the baseline corresponds to the depth at which each potential was obtained. The amplitude of the evoked waveform, at a fixed latency (solid vertical line in *a, d*), is plotted as a function of depth in *b* and *e*. Zero depth was taken arbitrarily as 70 μ m above the point of maximum negativity, corresponding approximately to the hippocampal fissure. A spot of dye was iontophoretically ejected from the recording electrode at the end of each experiment. This gave a reference point enabling the position of the cell bodies to be accurately aligned with the corresponding depth profile (*c, f*). All animals were aged between 18 and 21 d and were anaesthetised with Equithesin.

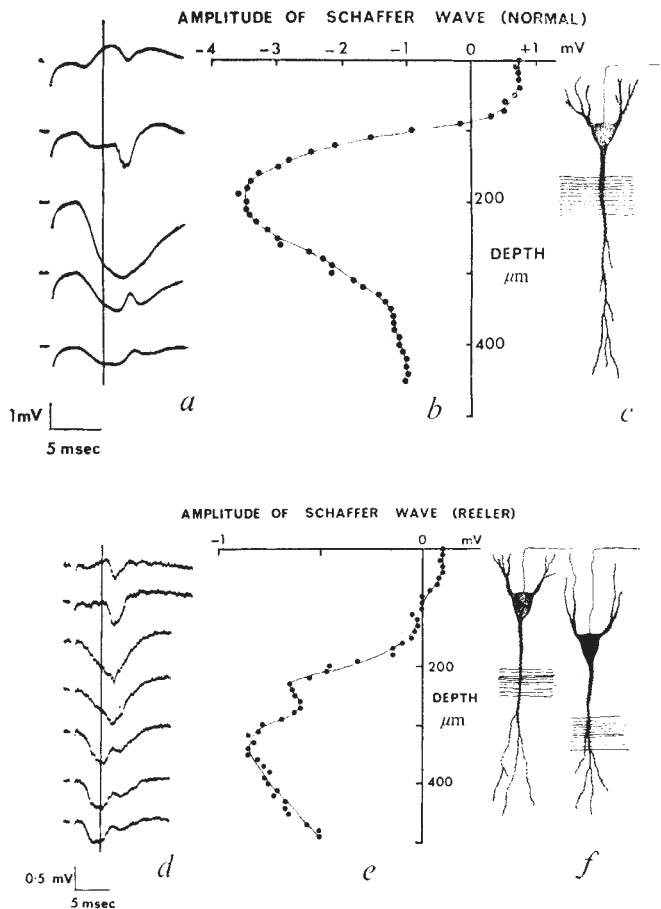


Fig. 3 Depth profiles of Schaffer waves in normal (*a-c*) and reeler (*d-f*) littermates. Samples of waveforms elicited by stimulating the Schaffer collaterals are displayed in *a* and *d*. The amplitude of the evoked waveform at a fixed latency (solid vertical line) is plotted as a function of depth from the hippocampal surface (*b* and *e*). The relative positions of the pyramidal cell population and Schaffer fibres are illustrated in *c* and *f*.

evoked potential, at a fixed latency, as a function of depth. In Fig. 2*b*, a profile of this type, obtained by stimulating the perforant path in a normal mouse, is plotted together with representative responses at various depths (Fig. 2*a*). The relative position of the granule cells is illustrated diagrammatically in Fig. 2*c*.

The depth profile of the perforant path response in the reeler (Fig. 2*e*) is essentially similar to that seen in the normal animal. As in the normal, the evoked potential is negative in the molecular layer, superficial to the region of scattered granule cells, and its polarity reverses as the electrode advances to the cell body region. This result, which was consistently obtained in reeler, shows that perforant path terminals occupy the same position in the reeler as they do in the normal animal. Two additional lines of evidence indicate that displaced granule cells establish synaptic contact with the perforant path by extending their dendrites into the molecular layer. First, records were obtained from single cells in the dentate area; in reeler, cells responding to stimulation of the perforant path could be found throughout the hilar region, whereas in normal mice such neurones were confined to the granule layer. Secondly, we compared Golgi impregnated material in normal and reeler mice. In the normal animal, granule cells were restricted to the granule layer; in reeler, granule cells were frequently seen in the hilus, with abnormally elongated dendritic arborisations, sometimes extending into both upper and lower blades of the molecular layer (Fig. 1*d*).

Pyramidal cells of region CA1 were activated by stimulation of the Schaffer collaterals. In the normal animal, the peak of the

synaptic region occurs approximately 100 μm below the pyramidal layer (Fig. 3*b, c*). In reeler, the pyramidal cell layer is split into two sublayers, separated by 100 μm (Figs 1*b* and 3*f*). The depth profiles obtained from reeler contained a double peak (Fig. 3*e*), showing that Schaffer collaterals form synaptic contacts with the dendritic shafts of pyramidal cells in two distinct layers, separated by the same distance as the cell body layers. This suggests that a different mechanism is at work in maintaining the integrity of the Schaffer projection from that discussed above for the perforant path. Instead of the post-synaptic cells extending their dendrites, the afferent fibres adjust their course to innervate the appropriate dendritic section of the displaced cells, either by means of a diversion of part of the Schaffer bundle to lower than normal depths, or by collateral sprouting.

The development of the hippocampus involves a number of events occurring in sequence. Autoradiographic analysis¹³ has shown that pyramidal cells are generated mainly between the 12th and 15th day prenatally, while granule cells mitose from the 16th prenatal day until the end of the third postnatal week. From the germinal layer, neurones migrate to their eventual destination and form laminar aggregates before making functional connections between one neurone population and another. In the rat, synaptic connections reach adult levels of efficacy by the end of the third postnatal week¹⁴. The events which lead to cellular disarray in the reeler brain presumably occur during, or subsequent to cell migration¹⁵. The site of the primary lesion of mutant gene action is not known; for example the migrating cells may be deprived of a genetic instruction to reach a given site, or they may be devoid of a surface property which facilitates aggregation once they have arrived; cortical cells cultured *in vitro* from reeler mice do not aggregate to form the laminar arrays characteristic of cells from normal littermates^{16,17}.

Our present findings show that in reeler mice, orderly synaptic connections can be made between neurone populations in the hippocampus in spite of a genetic lesion affecting a prior developmental stage. When a granule cell is displaced in the hilar region of the dentate area, the dendritic tree of the cell is extended to the region traversed by the perforant path fibres. This suggests that the dendrites of displaced cells continue to grow until specified synaptic contacts are made. An alternative kind of adjustment to spatial disorganisation is seen in region CA1, where malpositioned pyramidal cells receive a normal Schaffer input. Analysis of the depth profiles suggests that a segment of the dendritic shaft at a given distance from the cell body has a high affinity for Schaffer fibres, and that when these segments are misaligned, afferent fibres are diverted, or form collaterals, to make synaptic junctions with the appropriate region of the postsynaptic cell. Thus, there is a high safety factor in the overall developmental programme for the hippocampus which ensures that the effects of a previous error are minimised.

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Different spike mechanisms in axon and soma of molluscan neurone

STUDIES with a few invertebrate preparations have suggested different ionic mechanisms for spike generation in the soma and axon of the same neurone. Iwasaki and Satow¹ found that somatic spikes in the crayfish eyestalk *X* organ had a mixed Na–Ca dependency, while the extracellular axon spikes disappeared in Na-free or tetrodotoxin (TTX)-containing solutions. Wald² recorded attenuated axon spikes in hyperpolarised somata of snail ganglion cells and showed that they varied in amplitude with external sodium concentration but, curiously, were not blocked by TTX. In voltage-clamp experiments with the left pleural giant cell in *Aplysia*, Kado³ found that early inward currents in the axon-hillock region were much more sensitive to TTX than early inward currents in the opposite end of the soma, suggesting a greater Na-dependency of the axon spike.

In none of these studies, however, were action potentials recorded with intracellular electrodes in the axon at a large distance from the cell soma. We have been able to record from the axon of the right giant (R2) cell in the visceral ganglion of *Aplysia* at a distance of 3–4 cm from the soma. Our measurements indicate that the axon length constant is about 1 cm, so the axonal and somatic recording sites are electrically remote. This direct recording technique confirms that the axon spike is mainly sodium dependent, while the somatic spike has both sodium and calcium components^{4–6}.

In all experiments the visceral ganglion together with the right (dorsal view) visceropleural connective was dissected from the animal and placed in a small-volume recording chamber containing normal saline. The preparation was then treated with a 1% solution of pronase (Calbiochem) in normal saline for 15–20 min to facilitate impalement through the epineurium. The microelectrodes used had resistances from 3–8 MΩ. Standard intracellular recording techniques were used, and all experiments were performed at room temperature.

The R2 axon is visually indistinguishable from the other axons in the connective because of its small diameter (35 μm) and lack of pigmentation⁷. Thus, we usually identified the axon by stimulating the soma with regular (1 s⁻¹) pulses while searching across the right connective with a recording electrode at least 3 cm distal to the soma. Identification of the axon was made when both a 40–60 mV resting potential and 70–90 mV spike with a constant delay after the somatic spike were recorded with the axon electrode. The conduction velocity from soma to axon was typically 1 m s⁻¹. In some experiments, stimuli were applied to the right connective with a suction electrode. This method was more convenient than attempting to place a second intracellular stimulating electrode in the axon. The suction electrode also enabled us to depolarise the axon more than was possible with a subtracting circuit for stimulating through the recording

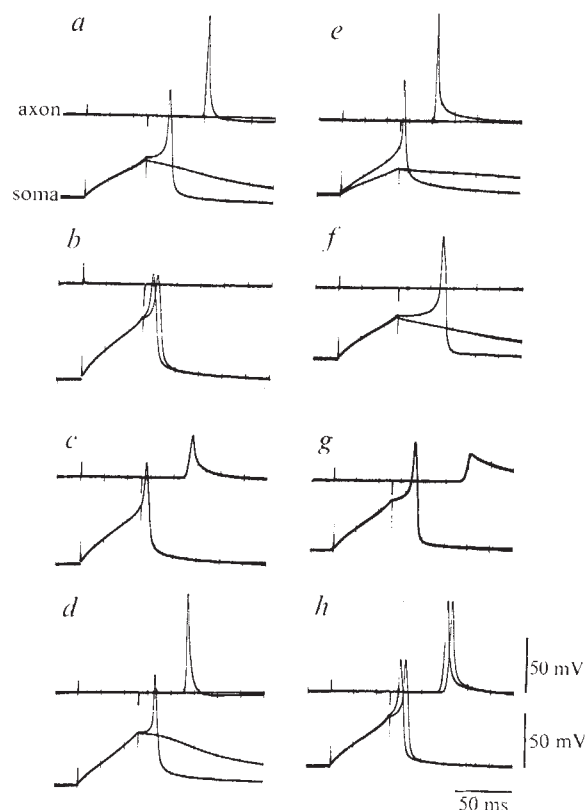


Fig. 1 Responses to somatic stimulation measured in soma and axon at 3 cm from soma. Two sweeps superimposed in most pictures. *a*, Normal saline; *b*, Na-free (Tris) solution; *c*, 2 min after perfusion with normal saline again; *d*, normal saline, 5 min later; *e*, normal saline; *f*, Na-free, Ca 340 mM; *g*, 2 min after perfusion with normal saline again; *h*, normal saline, 5 min later. All data from the same cell.

electrode. The R2 cell axon was apparently the only one from which we could record intracellularly, as no smaller spikes or large variations of conduction velocity were seen with antidromic stimulation.

Solution changes were made by perfusing about 40× the chamber volume of each solution. The normal saline used had the composition: NaCl 494 mM, KCl 11 mM, CaCl₂ 11 mM, MgCl₂ 19 mM, MgSO₄ 30 mM, Tris-HCl (pH 7.7) 10 mM. Sodium-free saline had the same composition except that 540 mM neutralised Tris was substituted for sodium. High-calcium saline was made by replacing the NaCl with 340 mM CaCl₂ and substituting MgSO₄ with MgCl₂ to prevent precipitation of CaSO₄.

The mixed Na–Ca dependency of the somatic action potential in the R2 cell has been shown previously^{4–6}. The ionic dependency of the axon spike is compared with that of the somatic spike in Fig. 1. The top trace in each part is the axon membrane potential measured at about 3 cm from the soma, and the bottom trace is the somatic membrane potential. Depolarising stimuli were applied to the soma through a second intracellular electrode. Figure 1*a* was obtained in normal saline, and shows two superimposed sweeps, one in which the stimulus was subthreshold, and one in which it was suprathreshold. The axon spike was 87 mV, and the somatic spike was 93 mV, measured from resting. Figure 1*b* shows the blockage of the axon spike which was seen after about 2 min in Na-free (Tris) medium, while the somatic spike was still 88 mV, measured from resting; Figure 1*c* was taken after the start of perfusion with normal saline again, and shows a partial recovery of the axon spike and Figure 1*d* shows the final recovery of the axon spike to 88 mV, measured from resting.

To further test the axon's dependency on sodium we treated the preparation with 30 μM tetrodotoxin (TTX: Calbiochem).

TTX has been shown to block selectively the active sodium conductance in a number of excitable membranes⁸, and would be expected to block the axon spike if it were mainly dependent on sodium. After 10–15 min, TTX completely and reversibly blocked the axon spike in response to somatic stimulation, while having little effect on the soma spike.

If the axon membrane has a calcium channel like that in the soma, then it should be possible to produce axon spikes in a solution in which all the sodium is replaced with calcium (the somatic action potential is larger in such a solution than in normal saline). Figure 1e–h, shows that we could not produce axon spikes in high-Ca solution by somatic stimulation. Figure 1e was obtained in normal saline, and shows two superimposed sweeps, one with a subthreshold stimulus. Figure 1f shows the blockage of the axon spike after about 2 min in Na-free, Ca 340 mM solution, while the soma spike was 108 mV. Figure 1g shows a partial recovery after the start of perfusion with normal saline, and Figure 1h shows the amount of recovery about 5 min later.

These experiments demonstrated that the axon could not conduct action potentials in Na-free solutions or in the presence of TTX. It was possible, however, that the axon membrane might still be excitable in Na-free solution, if a depolarising stimulus were applied close enough to the recording area to affect it directly. To test this possibility we stimulated the axon through a suction electrode applied to the cut end of the right connective, with a chlorided silver wire just outside the end of the electrode serving as the return path for the current. The recording electrode was placed in the axon at about 0.5 cm from the suction electrode. With this method of stimulation the axon spike was again blocked by replacement of external sodium with Tris. Within 5 min after perfusion with Na-free solution, depolarisations of up to 30 mV produced no regenerative response. Partial recovery of the spike was seen upon reperfusion with normal saline. The antidromic axon spike was also blocked by 30 μ M tetrodotoxin in the external solution. To test the possibility that the axon membrane might have a calcium channel like that in the soma, we repeated the high-calcium experiment shown in Fig. 1e–h, with direct axonal stimulation using a suction electrode. Replacement of external sodium with calcium completely blocked the axon spike within 5 min. This experiment indicated that, when external sodium was replaced with Tris or calcium, the R2 axon was not only incapable of conduction, but was also inexcitable even with direct stimulation.

The absence of an axon spike in Na-free solution when the soma was stimulated (Fig. 1b and f) could have been due to conduction block in the axon. The partial spike seen upon reperfusion with normal saline (Fig. 1c and g), however, indicated that the amplitude of the axon spike when present was strongly dependent on external sodium. The blockage of the axon spike when external sodium was replaced with calcium showed that the axon membrane does not have a specialised calcium channel like the soma^{4–6}. In addition these experiments suggested that no large calcium current goes through the axonal sodium channel during the action potential⁹.

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Regulation of catecholamine synthesis in the rat brain *in vitro* by cyclic AMP

EVIDENCE that catecholamines stimulate the production of adenosine cyclic-3',5'-monophosphate (cyclic AMP) in the pineal gland¹, superior cervical ganglia² and both in brain slices^{3,4} and homogenates⁵ has suggested that cyclic AMP has an important postsynaptic function in both peripheral and central adrenergic neurotransmission. Evidence has also been presented in the hypogastric nerve–vas deferens preparation⁶ and adrenal medulla⁷ that dibutyl cyclic AMP increases the release of noradrenaline, suggesting a presynaptic role for cyclic AMP in modulating catecholamine metabolism in adrenergic terminals. In the central nervous system, adenylate cyclase and phosphodiesterase are, to a considerable extent, associated with the isolated nerve-ending (synaptosomal) fraction⁸ in which putative neurotransmitters are primarily localised. Thus it was of interest to determine whether cyclic AMP might also stimulate catecholamine turnover in the brain, especially in synaptosomes prepared from brain homogenates.

Recent experimental evidence demonstrates that dibutyl cyclic AMP can increase the formation of dopamine from labelled tyrosine when incubated with brain slices⁹ or synaptosomes^{10,11} prepared from the corpus striatum. We report here, a stimulation of tyrosine hydroxylase activity in rat–brain synaptosomes incubated with cyclic AMP derivatives; this effect can also be produced by cyclic AMP in a soluble preparation of tyrosine hydroxylase and is associated with changes in the kinetic parameters of tyrosine hydroxylation suggestive of an allosteric activation of the rate-limiting enzyme in catecholamine synthesis.

Tyrosine hydroxylase activity was assayed in crude synaptosomal fractions of rat striatal homogenates by a procedure (Table 1) which utilises the natural endogenous pteridine cofactor of tyrosine hydroxylase¹². The rate of evolution of labelled CO₂ resulting from the decarboxylation of newly formed ¹⁴C-dopa was linear for at least 20 min and varied directly with the quantity of tissue present in the assay. The

Table 1 Effects of cyclic nucleotides on tyrosine hydroxylase activity in striatal homogenates

Compound added	% of control tyrosine hydroxylase activity $\pm$ s.e.m.
8-Bromo-cyclic AMP	183 $\pm$ 7*
Dibutyl cyclic AMP	158 $\pm$ 3*
Monobutyl cyclic AMP	152 $\pm$ 2*
8-Methyl-thio-cyclic AMP	136 $\pm$ 4*
Cyclic AMP†	79 $\pm$ 3*
Dibutyl cyclic GMP	84 $\pm$ 1*
Na butyrate	99 $\pm$ 3

Tyrosine hydroxylase activity was determined in a preparation of striatal synaptosomes¹² preincubated for 5 min in the presence or absence of cyclic nucleotides (1 mM) or Na butyrate (2 mM) and incubated for a further 20 min with a saturating concentration of L-¹⁴C-L-tyrosine (20 μ M), in a Krebs–Ringer–phosphate medium at 37°C, pH 6.7. Mean control activity $\pm$ s.e.m. = 20.3 $\pm$ 0.3 nmol ¹⁴CO₂ per g wet weight h⁻¹ and is represented as 100%.

*Significant difference by Student's *t* test (*P* < 0.01) of the difference between enzyme activity in control compared with treated tissues for at least 9 to 12 experiments.

†In the presence or absence of theophylline (1.0 mM).

activity was stereospecific for the L-isomer of tyrosine and could be inhibited by low concentrations of 3-iodotyrosine or of dopamine, or pretreatment with intracranially administered 6-hydroxydopamine. Protein was determined by the method of Lowry *et al.*¹³ with bovine serum albumin as a standard.

Table 1 shows that incubation of striatal homogenates with either dibutyl or monobutyl cyclic AMP, which are poor substrates for phosphodiesterase and to which membranes are relatively permeable, produced similar stimulation of activity, while cyclic AMP, which is less able to penetrate cell membranes¹⁴, produced a small inhibition, even when coincubated with the phosphodiesterase inhibitor theophylline (1 mM). Enhancement of the tyrosine hydroxylase activity was also found with 8-bromo-cyclic AMP and 8-methyl-thio-cyclic AMP, further suggesting that the stimulation might be mediated by the cyclic AMP moiety of these derivatives. The stimulation of tyrosine hydroxylase activity induced by dibutyl cyclic AMP was not due to the butyrate moiety since incubation with sodium butyrate had no effect, and a small decrease was produced with dibutyl cyclic GMP. The stimulatory effect of dibutyl cyclic AMP did not occur distal to the tyrosine hydroxylation step since there was no alteration in the rate of formation of ¹⁴CO₂ when carboxy-¹⁴C-D,L-dopa (0.1 mM) was substituted for tyrosine. When the concentration of dibutyl cyclic AMP in the incubation medium was altered, significant dose-dependent increases of tyrosine hydroxylase activity were observed, from 50 μ M (about 14%) to a maximum (60%) at 1.0 mM of cyclic nucleotide when striatal synaptosomes were used. The hydroxylase activities in P₂ synaptosomal fractions^{12,15}, prepared from various brain regions were measured in the presence of dibutyl cyclic AMP (1 mM); the hydroxylation of tyrosine was increased to a similar extent in striatal (57 $\pm$ 3%) and cerebral cortical (51 $\pm$ 2%) synaptosomes, and an even greater stimulation occurred in the brainstem (85 $\pm$ 4%). Thus, dibutyl cyclic AMP seems to stimulate the rate of catecholamine synthesis in isolated nerve endings in several regions of the rat brain.

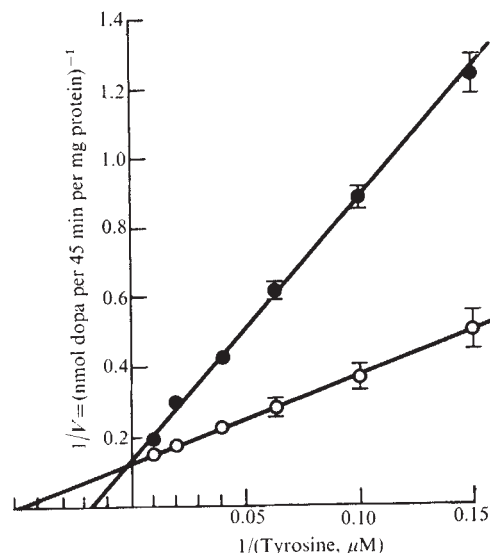


Fig. 1 Effect of cyclic AMP on the K_m for tyrosine of striatal tyrosine hydroxylase. Soluble tyrosine hydroxylase activity was assayed in a 100,000g supernatant of striatal homogenates by a modification of the method of Shiman *et al.*¹⁷. Tissues (50–70 μ g protein) were pre-incubated for 5 min in the presence or absence of cyclic AMP (10 μ M) and incubated for a further 45 min in 1.0 ml of medium containing: DMPH₄ (1 mM), sheep liver pteridine reductase, NADPH (1 mM), catalase (1,100 U), sodium acetate buffer, pH 6.0, and various concentrations of ³H-3,5-L-tyrosine. Tyrosine hydroxylase activity was linear with time and protein concentration under the conditions used for the determination of K_m . Each value is the mean $\pm$ s.e.m. of at least six determinations. The lines drawn giving the best fit produced an apparent K_m for tyrosine in cyclic AMP-treated tissues of $22.3 \pm 2.7 \mu$ M, which is significantly different ($P < 0.01$) from untreated controls of $53.6 \pm 3.9 \mu$ M, with linear regression coefficients $r > 0.95$. $\circ$, 10 μ M cyclic AMP; $\bullet$, control.

Table 2 Effects of cyclic AMP on the kinetic parameters of soluble, striatal tyrosine hydroxylase

	K_m —DMPH ₄ (mM)	K_i —dopamine (mM)
Control	0.62 ± 0.05	0.09 ± 0.02
Cyclic AMP (0.01 mM)†	$0.08 \pm 0.02^*$	$0.64 \pm 0.04^*$

According to the assay procedure described in Fig. 1, the K_m for DMPH₄ was determined by the method of Lineweaver and Burke¹⁸, at a tyrosine concentration of 0.1 mM and six DMPH₄ concentrations ranging from 10^{-5} to 10^{-3} M. Each value is the mean $\pm$ s.e.m. of the intercepts generated from six separate lines. The K_i was determined by the method of Dixon¹⁹, at three DMPH₄ concentrations of 10^{-4} , 10^{-5} , 10^{-6} M, a constant L-tyrosine concentration of 10^{-4} M and seven dopamine concentrations ranging from 10^{-5} to 10^{-3} M.

*Indicates significant difference from controls at $P < 0.01$.

†Cyclic AMP was added to the assay mixture 5 min before the initiation of the reaction by addition of substrate L-tyrosine.

Since cyclic AMP derivatives might cause an alteration in membrane permeability of neural tissues, we attempted to determine whether the observed stimulation of synthesis could be explained by an alteration of the uptake of substrate tyrosine into synaptosomes. This is probably not the case, since the uptake (0.5 to 20 min) of labelled tyrosine (20 μ M) into striatal synaptosomes, selectively recovered on Millipore filters¹⁶, was not altered by incubating with dibutyl cyclic AMP (up to 5 mM). Furthermore, in synaptosomes preincubated with dibutyl cyclic AMP and subsequently washed, tyrosine hydroxylase activity was stimulated, even though the dibutyl cyclic AMP was not present in the incubation medium. These findings suggest that the stimulation of tyrosine hydroxylase activity is not a result of an increased uptake of labelled precursor.

Another possibility would be a direct effect of cyclic AMP derivatives on tyrosine hydroxylase which could involve either an increased affinity for tyrosine or the pteridine cofactor, or a decreased affinity for end-product catecholamines. We then investigated the effects of dibutyl cyclic AMP and cyclic AMP on the activity of soluble tyrosine hydroxylase prepared from the rat striatum in incubations which included a partially purified sheep liver pteridine reductase system¹⁷ and subsaturating concentrations of both 3, 5-³H-L-tyrosine (10 μ M) and 2-amino-4-hydroxy-5,6,7,8-tetrahydropteridine (DMPH₄) (0.1 mM). Activity of soluble tyrosine hydroxylase was increased by about 40% and 57% with 0.1 mM dibutyl cyclic AMP and cyclic AMP, respectively, while sodium butyrate had no effect. This activation was no longer evident, however, when saturating concentrations of tyrosine and cofactor were used. By Lineweaver–Burke analysis¹⁸, the apparent K_m for tyrosine was significantly decreased in the presence of 10 μ M cyclic AMP (Fig. 1) without any change in V_{max} . Finally, when we applied Dixon kinetic analysis¹⁹, a seven-fold increase in the inhibitory constant (K_i) for dopamine was observed with cyclic AMP (Table 2), suggesting a decreased affinity of soluble tyrosine hydroxylase for dopamine. In the presence of cyclic AMP, a decrease in the K_m for DMPH₄ (Table 2) of seven to eightfold was also obtained without a significant change in V_{max} . A possible interpretation of these findings with soluble tyrosine hydroxylase is that cyclic AMP produces an allosteric activation of striatal tyrosine hydroxylase which is mediated by increased affinities of the enzyme for substrate (tyrosine) and cofactor and a decreased affinity for the end-product inhibitor dopamine.

The significance of such a mechanism in the regulation of catecholamine synthesis *in vivo* requires further investigation. Cyclic AMP, however, may play a role in the activation of tyrosine hydroxylase activity observed during increased activity

in both peripheral and central adrenergic neurones. Recent experiments have demonstrated that electrical stimulation of the guinea pig hypogastric nerve–vas deferens preparation²⁰, as well as central dopaminergic²¹ and noradrenergic neurones²², causes an allosteric activation of tyrosine hydroxylase. This activation seems to be mediated by an increased affinity for both substrate and pteridine cofactor and a decreased affinity for end-product inhibitor^{20–22} which is similar to that observed for cyclic AMP in the present experiments. In view of this theory and the observations that both electrical stimulation and depolarising agents can produce increased concentrations of cyclic AMP in brain slices²³, one might postulate that cyclic AMP is partly responsible for an allosteric activation of tyrosine hydroxylase during neuronal depolarisation. Finally, the possibility that the mechanism underlying this allosteric effect involves activation of a cyclic AMP-dependent protein kinase and subsequent phosphorylation of tyrosine hydroxylase or some activator of this enzyme is under investigation.

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Cadaverine in the brain of axenic mice

CADAVERINE has been shown to be present in the central nervous system (CNS) (refs 1, 2 and personal communication from M. Wiechmann). Its concentration in the whole brain varies during behavioural sleep in mammals and during hibernation in molluscs^{1,2}. It is a precursor of piperidine, which has been linked with some functional stages of the CNS. Cadaverine is the source of much of the piperidine excreted in the urine³. Cadaverine has been considered a non-metabolite of the brain, and its presence in the body was believed to be due almost entirely to bacterial decarboxylation of lysine in the intestine⁴. In slices of mouse brain, exogenous cadaverine accumulates against a concentration gradient and can reach a concentration ten times greater in the tissue than in the surrounding medium⁴. The question has been therefore: does the cadaverine in the brain originate from an exogenous source; is it formed and resorbed in the intestine? Assaying cadaverine in axenic mice, we have now found that this is not the case, and that there is an endogenous source of cadaverine in the mouse.

Ten 3-month-old mice of each of the following strains were used: C57BL/6 (pathogen free colony of this department); CD-1 COBS (Caesarean originated barrier sustained, Charles River), and CD-1 germ-free (Charles River). For the final 14 d, the CD-1 COBS mice were fed the same diet as the germ-free stock. All animals were killed when active, that is moving in the cage, grooming and so on.

The mice were decapitated, the whole brain and blood samples were homogenised in perchloric acid, and the homogenate was dansylated and separated by thin-layer chromatography on silica gel. DANS-piperidine and *bis*-DANS-cadaverine TLC fractions were identified mass spectrometrically by their molecular ions (*m/e* 318 and *m/e* 568) and quantified by comparing their integrated ion currents with those of added internal standards of DANS-pyrrolidine (*m/e* 304) and *bis*-DANS-hexamethylenediamine (*m/e* 582) (for details and discussion of the method see refs 2 and 5).

Table 1 summarises our results. In all three groups of mice, cadaverine was found in both the brain and blood. The differences among individual mean values were not significant. The concentrations were measured as quantities more than 10 times higher than their blanks.

Table 1 Concentrations of cadaverine in the whole brain and blood of pathogen-free, COBS and germ-free mice

Mouse strain	Brain	Blood
C57BL/6	0.30±0.07 (7)	0.24±0.07 (7)
CD-1 COBS	0.20±0.06 (10)	0.22±0.06 (10)
CD-1 Axenic	0.55±0.17 (10)	0.27±0.12 (10)

Figures are in 10⁻¹² mol mg⁻¹ wet tissue, mean ± standard error. The numbers of measurements are given in parentheses.

The different origins and environmental conditions of tested animals had no apparent effect on the concentrations of cadaverine in their brain and blood. The lack of microbial flora in the germ-free mice also had no apparent effect on their brain and blood cadaverine levels. In contrast, there was significantly more piperidine in the blood of COBS mice (1.10±0.23 pmol mg⁻¹) than in that of germ-free mice (0.19±0.05 pmol mg⁻¹; *P*<0.01). It seems therefore that the cadaverine concentration in both the brain and blood of active mice is kept constant, irrespective of the formation of cadaverine by bacteria in the intestine. The increased supply of exogenous cadaverine is compensated for by its conversion to piperidine, without allowing any appreciable increase in cadaverine in brain or blood.

From our findings we make the following conclusions. (1) There is a source of cadaverine in mice that is independent of the bacterial decarboxylation of lysine in the intestine; (2) both the blood and brain concentrations of cadaverine in active mice

seem to be tightly controlled and are maintained at a level which does not depend on inflow of cadaverine from the intestine and (3) the concentration of cadaverine in the blood is probably maintained by the conversion of cadaverine into piperidine.

It seems therefore that there are two pools of cadaverine in mice. One provides both the blood and brain with an amount which might have some physiological importance which is unknown at present. The other pool, originating from bacterial decomposition of the food in the intestine, seems to be efficiently transformed into other compounds, one of them piperidine, and eliminated from the body.

There are indications that piperidine might be involved in functions of the central nervous system, particularly hibernation and sleep. Its concentration in the brain and blood varies during hibernation and behavioural sleep⁶⁻⁸, and it has relatively strong inhibitory effects on certain molluscan cholinergic neurones⁹. Our results suggest that cadaverine in the brain and blood of mice could participate in this process.

It is interesting in this context that the so called 'pink spots' observed on chromatograms of the urine of schizophrenic patients were considered to be due to the presence of monoacetyl- and monopropionylcadaverine¹⁰. If our findings with axenic mice are relevant for humans, the presence of large amounts of anomalous metabolites of cadaverine in the urine could reflect not only the altered dietary conditions of schizophrenic patients as suggested¹⁰, but may also indicate a failure of the normal catabolism of cadaverine in their central nervous system.

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Foetal origin of transferrin in mouse amniotic fluid

THE measurement of specific foetal products in amniotic fluid collected after amniocentesis offers an obvious approach to the foetus *in utero*. The proteins in mouse

amniotic fluid consist of three transferrins (Trf1, 2 and 3), five α foetoproteins (α FP 1-5) and albumins, the proportions of which change during gestation^{1,2}. The changes in composition directly parallel those observed in foetal serum, except that they occur 24 h later in the amniotic fluid. No comparable alteration is observed in maternal serum patterns. Transferrin 3 constitutes the major transferrin band both in late-stage fetuses and in adults, and so could be derived from either the foetus or the mother. We have investigated the origin of transferrin 3 in mouse amniotic fluid, using a genetic difference at the transferrin locus³ as a marker.

Reciprocal embryo transfers were made between inbred C3H/Bi/McL and randomly bred Q-strain mice. Blastocysts were flushed from donor uteri 3.5 d *post coitum*, and injected into the uteri of pseudopregnant recipients 2.5 d *post coitum*⁴. Plasma was collected from donor females (by severing the carotid arteries) at transfer and from recipient females when autopsied on day 18 of gestation. Foetuses were dissected from the uterus, with embryonic membranes intact, and amniotic fluid sampled by insertion of a 26-gauge needle directly into the amniotic cavity. Foetal plasma was collected in heparinised capillary tubes from an incision made in the external carotids. Fluids were analysed for protein on polyacrylamide slab gel electrophoresis⁵. Protein concentration was determined with the Lowry⁶ technique, using bovine serum albumin as standard.

All Q donors and recipients proved to be Trf^b/Trf^b, and C3H Trf^a/Trf^a. Analyses were carried out on six C3H embryos on Q recipients, and three Q embryos in C3H recipients, and the transferrin type of the foetus proved in every case to resemble that of the donor strain (Fig. 1A). Amniotic fluid transferrins were identical in electrophoretic mobility to those of the fetuses with which they were associated. Mixtures of plasma of recipient females and transferred foetuses or their amniotic fluids resolved two Trf 3 bands, while mixtures of the donor plasmas with foetal plasmas or amniotic fluids yielded one only (Fig. 1B), clearly demonstrating the foetal origin of the Trf 3 transferrin in the amniotic cavity.

Since Gustine and Zimmerman¹ have already shown a foetal origin of the five α foetoproteins and Trfs 1 and 2, our result indicates that all mouse amniotic fluid proteins, with the possible exception of the albumins, are of foetal origin. The similarities between the protein patterns in amniotic fluid and foetal plasmas, and the absence of many maternal-type proteins in either fluid, support this. The yolk sac, which is one site of α foetoprotein synthesis⁷, completely surrounds the amnion in the mouse, and may be important for transfer of the other proteins as well, if not for their synthesis. The increase in sialyl transferase activity in the foetal liver coincides with the increase in number of sialic acid residues in the foetal plasma α foetoprotein, while the comparable changes in yolk sac sialyl transferase activity and in amniotic fluid α foetoprotein occur about one day later^{1,7}. This suggests that the α foetoprotein in foetal plasma may be mainly derived from the foetal liver and the α foetoprotein in amniotic fluid may be derived from the yolk sac.

The rapid changes in the volume and protein patterns of mouse amniotic fluid^{1,2,8} may be related to the short time spent in the uterus. Indeed, mouse amniotic fluid, perhaps by virtue of its proximity to the large and vascular yolk sac, has considerable similarities to marsupial yolk sac fluid, which is believed to play an important role in foetal nutrition⁹, and in which, like the mouse amniotic fluid, the transferrins are of foetal origin¹⁰. Although the placental attachments are quite different in mice and marsupials, in both species the yolk sac prevents the amnion from making contact with the uterine lumen and maternal circulation, unlike in man, where the amnion is in close contact with the uterine epithelium.

The obvious route by which a transferrin of foetal origin could pass into the amniotic cavity in mice is from the vitelline vessels across the exocoel and amniotic membrane (Fig. 2). It seems unlikely that transferrin proteins would diffuse from the foetal skin or alimentary canal into the amniotic cavity and the area around the umbilical stalk where the amnion is close to the allantoic vessels is small. In man, however, these other, perhaps less efficient, routes are the only ones available for transfer of foetal protein to the amniotic cavity, as the yolk sac remains small and rudimentary throughout development.

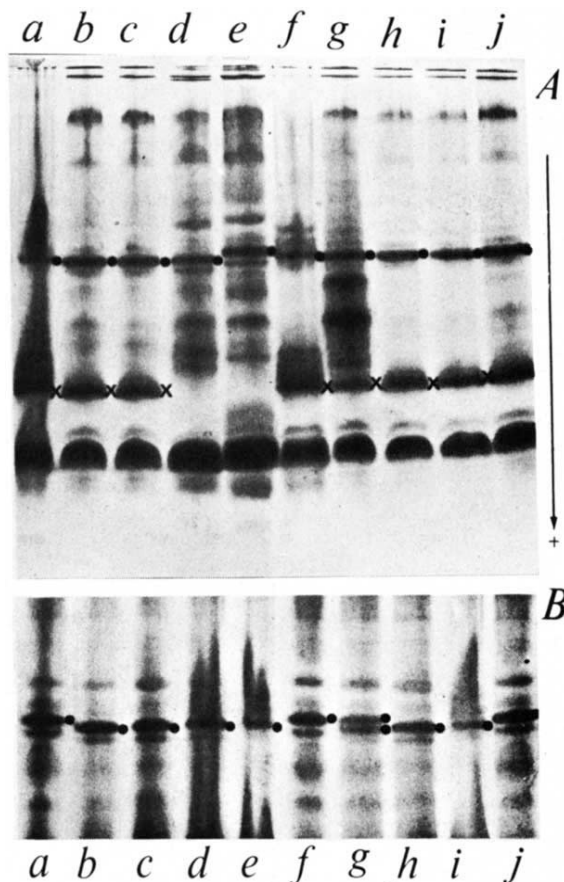


Fig. 1 Electropherogram of mouse plasmas and amniotic fluid. *A*, Replications *A* and *B*: C3H blastocysts ($\text{Trf}^{\text{H}}/\text{Trf}^{\text{A}}$) transferred to Q recipients ($\text{Trf}^{\text{B}}/\text{Trf}^{\text{B}}$). Wells from left to right: *a*, amniotic fluid; *b*, foetal plasma 1; *c*, foetal plasma 2; *d*, C3H donor plasma; *e*, Q recipient plasma; *f*, amniotic fluid; *g*, foetal plasma 1; *h*, foetal plasma 2; *i*, foetal plasma 3; *j*, foetal plasma 4. *B*, Mixtures of donor and recipient plasmas and amniotic fluid. Wells from left to right: *a*, recipient plasma Q ($\text{Trf}^{\text{B}}/\text{Trf}^{\text{B}}$); *b*, foetal plasma (C3H)+donor plasma (C3H); *c*, donor plasma C3H ($\text{Trf}^{\text{H}}/\text{Trf}^{\text{A}}$); *d*, amniotic fluid+donor plasma; *e*, amniotic fluid; *f*, recipient plasma; *g*, recipient+donor plasma; *h*, donor plasma; *i*, amniotic fluid; *j*, recipient plasma. Note that the only mix which yields two transferrins is that of donor and recipient plasmas: donor plasma mixed with its transferred foetal plasma or amniotic fluid gives only one transferrin. Gels were run at 30 mA, 100 V for 2.5 h, and stained in 0.025% Coomassie Blue+0.1% Amido Black in 25% methanol and 7% acetic acid for 45 min. Destaining was by diffusion for 3 h in 25% methanol and 7% acetic acid in water. 25 μl of amniotic fluid (3 mg ml^{-1}) and 2.5 μl of foetal plasma (20 mg ml^{-1}) and maternal plasma (45 mg ml^{-1}) were applied to each well. A tracking dye of bromophenol blue in 40% sucrose was used to colour the samples and show the boundary of the fastest moving components. The extra bands visible in the β globulin region (between transferrin and α foetoprotein) in some samples are due to free haemoglobin and haemoglobin/haptoglobin (Hb/Hp) bands resulting from haemolysis of those plasmas during collection.

●, Transferrin proteins; ×, α foetoproteins.

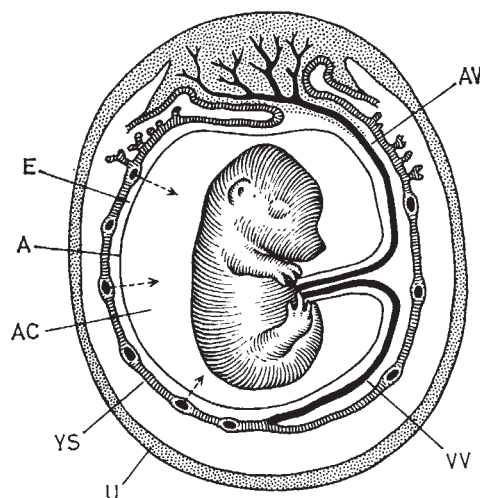


Fig. 2 The arrangement of foetal membranes in the mouse on day 15 of gestation, showing the likely route of transfer (arrows) of protein from the foetal circulation to the amniotic fluid. The orientation and relative proportions of the embryo are diagrammatic. E, Exocoelomic cavity; A, amnion; AC, amniotic cavity; VV, vitelline vessels; AV, allantoic vessels; YS, yolk sac splanchnopleur; U, uterus.

The composition and origin of human amniotic fluid components reflect this difference. Group-specific component and transferrin, unlike those in the mouse, have been shown to be of maternal origin¹¹⁻¹⁴, and the relative proportions of different serum proteins in amniotic fluid throughout pregnancy resemble those in maternal serum¹¹. Although the foetus makes some contribution^{13,16}, the only proteins for which a foetal origin has been unequivocally demonstrated are hexosaminidase A and B, arylsulphatase A, β galactosidase and α foetoprotein¹⁷⁻²¹. In man, the relative concentration of α foetoprotein in amniotic fluid and in foetal serum is 1:150 between 10 and 20 weeks¹¹, whereas in the mouse it is approximately 1:10 at days 11-18. Clearly, the foetal contribution to amniotic fluid is more substantial in mice than man.

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Liposomes can mimic virus membranes

CONSIDERABLE insight into mechanisms of membrane permeability and transport has been obtained from the study of simple lipid membranes. For the study of membrane-membrane interactions, however, such model systems have been used to a much lesser extent. If present ideas that biological membranes possess extended areas of lipid bilayer are correct, then it is probable that bilayer regions are involved in intermembrane phenomena such as adhesion and fusion, and it becomes reasonable to use simple lipid membranes to mimic such behaviour.

We set out to do this with respect to the membrane associated activities exhibited by many of the enveloped viruses. As the model virus, we used a modification of what has been described as a lipid dispersion, lipid vesicles, or liposomes, that is, single or multiple concentric shells of lipid bimolecular leaflets. The 'non-genetic' viral activities that are duplicated by the model virus membrane include attachment of the virus to the host cell, fusion of the viral membrane with the cytoplasmic membrane of the host cell, fusion of membranes of two or more host cells, and in the case of erythrocytes, haemolysis.

With respect to cell fusion, lysolecithin alone and in an oil emulsion has been used with success, although cell lysis is a problem^{1,2}. Very recently, mixtures of phosphatidyl choline and either phosphatidyl serine (PS) or phosphatidyl glycerol in the form of liposome dispersions have been shown to be effective without adverse influence on cell viability³. In a related study, it was shown that lipid from phospholipid vesicles is incorporated into mycoplasma membrane, although the mechanism was not unequivocally elucidated⁴.

Lipid vesicles were prepared in the usual manner⁵. Lipids were dissolved in chloroform-methanol (4:1) which was subsequently removed under reduced pressure. The dry lipids were suspended at 2.5 mg ml⁻¹ in sucrose (0.3 M), EDTA (10⁻⁴ M) and tricine (2.0 × 10⁻² M) at pH 7.8 (stock lipid dispersion). Liposomes used in haemolysis experiments were then sonicated with a probe type apparatus for a few minutes at room temperature. Vesicles used in fusion experiments were sonicated further (40 min at 24°C) to yield a population with a high proportion of unilamellar vesicles⁶. Haemagglutination, haemolysis and cell fusion were assayed using standard virological methods. The zeta potential of liposomes was determined by microelectrophoresis⁷. The lecithin used in this study, diisostearoylphosphatidyl choline (L), was prepared by reaction of the appropriate anhydride with glycerophosphoryl choline in the presence of triethylisostearate⁸. Lysophosphatidyl choline (LL) was prepared from this lecithin by hydrolysis with phospholipase A (ref. 9) and purified by ether precipitation. Stearylamine (SA, 99.5%) was purchased from Lachat Chemical Company.

Figure 1 depicts the relationship between the zeta potential of liposomes containing up to 2% SA and the haemagglutination

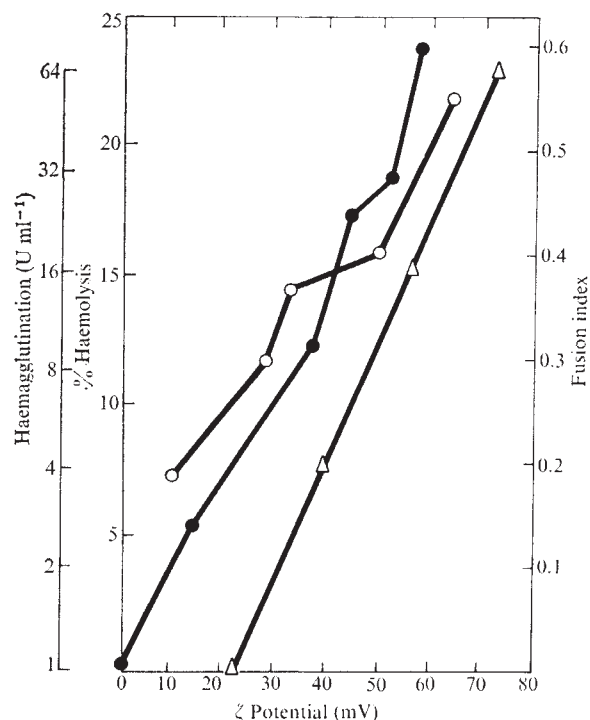


Fig. 1 Membrane activities are related to liposome surface charge. ○, Okada's fusion index for EATC¹⁰ for liposomes containing 25% LL and various amounts of SA (0.2–3.0%). ●, Haemolysis of sheep red cells by liposomes containing 20% LL and varying amounts of SA. △, Log₁₀ of the haemagglutination titre of liposomes (5 mg ml⁻¹) containing only lecithin and SA. All functions are plotted against the zeta potential of the corresponding liposomes. The haemagglutination titre of liposome dispersions was measured by the Salk pattern method¹¹ using a 0.5% (v/v) suspension of washed sheep red blood cells in 0.3 M sucrose buffered with 20 mM tricine (pH 7.6). For haemolysis experiments, equal volumes of a 10% suspension of the sheep cells in buffered sucrose and the stock liposome dispersion were mixed at 4°C and the cells were allowed to agglutinate. The temperature was then raised to 37°C. After 30 min, % haemolysis was determined by the method of Neurath and Sokol¹². Ehrlich ascites tumour cells for fusion experiments (EATC) were grown intraperitoneally in adult Swiss mice of both sexes¹⁰. The cells were then washed three times with Hank's balanced salt solution (BSS) to remove contaminating erythrocytes and suspended at a concentration of 10% (v/v) in 0.3 M sucrose buffered to pH 7.8 with 20 mM tricine-NaOH. 0.25 ml of lipid dispersion was added to 1 ml of a 10% EATC suspension. The incubation procedure for fusion was the same as that used in the haemolysis experiments. Fusion was assayed using methods described by Okada¹⁰.

(HA) titre of a dispersion containing 5 mg ml⁻¹ lipid. When plotted on a logarithmic unit scale, the relationship is linear up to 70 mV, above which value it would no longer be expected that the zeta potential would be an accurate measure of the surface potential. That the liposomes are the actual agglutinating agent rather than unincorporated SA is indicated by the following observations. First, when such dispersions are centrifuged at speeds sufficient to sediment liposomes but not SA micelles, 99% of the HA activity is found in the pellet. Second, the zeta potential of liposomes is proportional to the SA content and is stable for hours, indicating that there is little tendency for the SA to leave the liposomes.

Inclusion of LL into positively charged liposomes renders them active in the lysis of sheep erythrocytes. At constant surface charge density, the haemolysis rate increases with increasing LL content. Similarly, at constant LL values, the extent of haemolysis increases linearly with zeta potential. This is illustrated in Fig. 1. Neutral and negatively charged vesicles are devoid of haemolytic activity at the concentrations tested. We have compared the time course of liposome-induced haemolysis with that which has been reported for EDTA-treated Sendai virus and, aside from a scaling factor, the

relationships are virtually identical. In addition, there is a linear relationship between the concentration of liposomes (HA titre) and the logarithm of percentage haemolysis, the same kind of relationship that obtains with EDTA-treated Sendai virus¹³.

Liposomes capable of haemolysis also promote fusion of actively metabolising cells. Two animal cell types, EATC and KB, exhibit extensive cell fusion following treatment of cultures with L-LL-SA liposomes. Micrographs of representative examples of polykaryons of such cells are shown in Fig. 2. In our experience polykaryons produced by liposomes and Sendai virus are indistinguishable. As is the case with the fusion-promoting viruses, suspended cells treated with liposomes agglutinate at 4°C and begin to fuse as the temperature is raised. The fusion index for EATC increases with both LL content and positive surface potential of the liposomes. The latter relationship is shown in Fig. 1, the data in which represent fusion indices determined shortly (30 min) after liposome treatment. Although, as is the case with virus-induced fusion, there is some loss of cell viability, it is rather minimal. For example, 30 h after a 15 min treatment with liposomes containing 20% LL and 5% SA, fusion indices were $45 \pm 5\%$ for KB and rabbit kidney cells and 0.58 (Okada's index for cells in

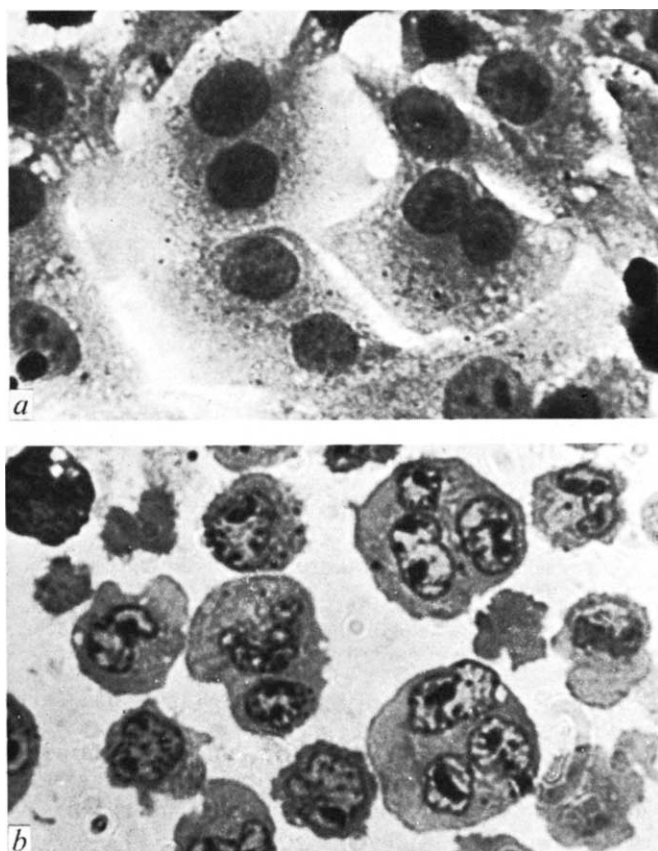


Fig. 2 Liposome-induced cell fusion. Polynuclear cells formed following treatment of *a*, KB cells monolayer and *b*, Ehrlich ascites tumour cells in suspension, with liposomes containing 20% LL, 5% SA and 75% L. 0.25 ml lipid dispersion was added to 1 ml of a 10% EATC suspension or to a KB monolayer in a 25 cm² culture flask containing 5 ml BSS. KB cells were grown in Eagle's minimal medium (EMM) and supplemented with 20% heat inactivated foetal calf serum in 25 cm² plastic flasks. The cultures were grown to confluence and maintained in EMM supplemented with 5% calf serum. For fusion experiments, the monolayers were washed with prewarmed BSS and treated with 0.25 ml of the liposome suspension at 37°C for 15 min. The cultures were then washed with BSS to remove excess liposomes and fresh maintenance medium added. For fusion assays, the cells were washed with BSS, treated with 0.02% EDTA in PBS for 1 min, fixed with 2% glutaraldehyde, stained with May-Greenwald Giemsa stain and polynuclear cells counted as described by Kohn¹⁴. EATC were treated as described in the legend to Fig. 1.

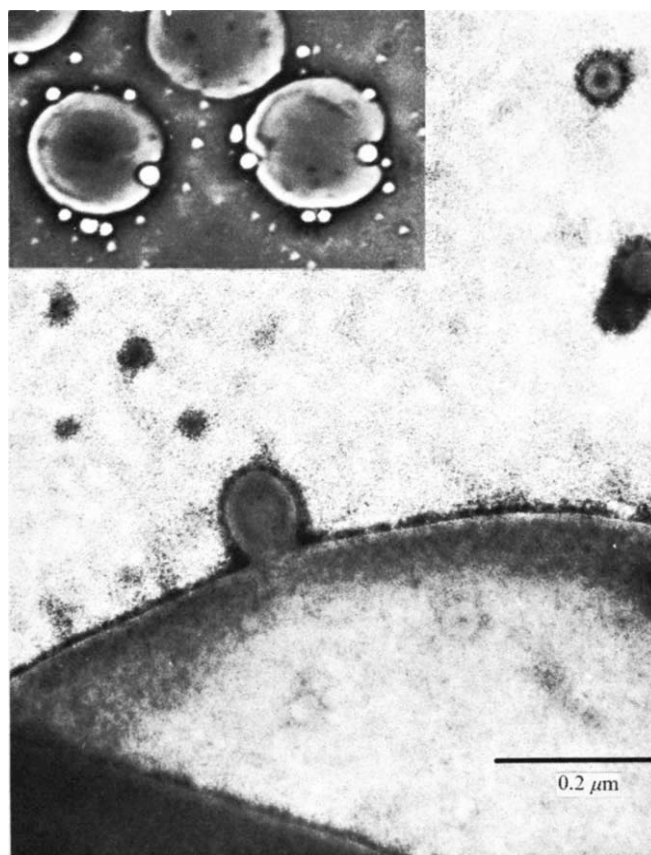


Fig. 3 Electron micrograph of a negatively stained preparation of human red cells treated with liposomes. A liposome is seen fusing with the erythrocyte membrane (Bar represents 0.1 μm). The inset is a phase micrograph of liposomes of the same composition adhering to the surface of red cells ($\times 900$). Freshly drawn human group O erythrocytes were washed five times with PBS and suspended at a concentration of 50% (v/v) in 0.3 M sucrose containing 0.1 M phosphate buffer (pH 7.3). The cells were then treated with unsonicated liposomes containing 20% LL, 5% SA and 75% L for 5 min at 37°C. A drop of the cell suspension was then lysed at the air-water interface and red cell ghosts floating on the surface were picked up on carbon strengthened parlodion grids. Without letting the preparation dry, the cells were fixed in osmium vapour for 5 min. The excess water was then removed from the grid surface with blotting paper and a drop of 2% potassium phosphotungstate (pH 6.5) was immediately placed on the grid. After 30 s the excess stain was removed with blotting paper and the preparation was allowed to air dry. The grids were then examined in a Hitachi 11-A electron microscope. For light microscopy, grids containing unstained liposome-treated ghosts were examined directly under the phase microscope.

suspension) for EATC. In all cases about 10% of the cells died (trypan blue exclusion) or detached from the substrate. This degree of cell loss accompanies each treatment; the extent of fusion, however, can be increased by multiple exposures of cells to the liposomes. Cell damage can be minimised by decreasing the liposome/cell ratio. In our experiments, each cell was treated with about 10^8 liposomes. This amount can be reduced by four orders of magnitude, and the fusion index is lowered by only 10%. At such low liposome levels, the decrease of cell viability in fusion experiments is reduced to practically nothing. These results are similar to those reported by Papahadjopoulos *et al.*³ for what they found to be the most efficient lipid mixture, L and PS. We have not obtained appreciable fusion with PS liposomes in our conditions.

By electron microscopy, enveloped viruses have been observed fusing with cell membranes¹⁵⁻¹⁹. It has been suggested that infection occurs by the same mechanism and, further, that binucleate cells are formed by the simultaneous fusion of the virus membrane with the membranes of two cells¹⁷. The fusion of liposomes with cell membranes may also be seen by electron

microscopy. Figure 3 is an electron micrograph of an L-LL-AS liposome in an intermediate stage of fusion with a human erythrocyte. A variety of such electron micrographs of cell membranes taken shortly after treatment with L-LL-SA liposomes reveals various stages from simple adhesion to nearly complete incorporation. The inset is a phase micrograph of liposomes of the same composition adhering to the surface of red cells.

The advantages of using simple artificial means to produce hybrid cells for genetic and other experiments have been noted³. The use of liposomes circumvents the necessity of finding a virus competent to fuse the cells of interest. As all cell membranes are negatively charged, the receptor for an 'artificial virus' of the type described here is universal. Model viruses also offer the opportunity of introducing foreign molecules into intact cells or into their membranes by incorporating such molecules into the aqueous or membrane phase, respectively, of the liposome²⁰.

The data presented here show that a lipid bilayer vesicle behaves like some virus membranes when a long chain cation and a lytic lipid are incorporated into it. It is possible therefore, that the basic requirements for viral membrane proteins that participate in infection activities, are to recognise specifically the host cell as, for example, would an antibody, and to disorder bilayer membranes in a manner similar to that of lyso compounds and detergents. We do not intend to imply either that viruses are positively charged or that lysolecithin is a necessary viral membrane component.

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closed spherical vesicles composed of a single lipid bilayer enclosing an aqueous space². Cultured mammalian cells will also incorporate these unilamellar vesicles without cytotoxic effects⁶. We present here data on the uptake of unilamellar vesicles by cells *in vitro* and the use of these vesicles as carriers to introduce biologically-active molecules into specific regions of the cell.

Incorporation of unilamellar vesicles with negative or positive surface charge by mouse 3T3 and L929 cells and human erythrocyte ghosts is shown in Fig. 1. Each cell type incorporated substantial numbers of vesicles (1 nmol lipid = 2×10^{11}

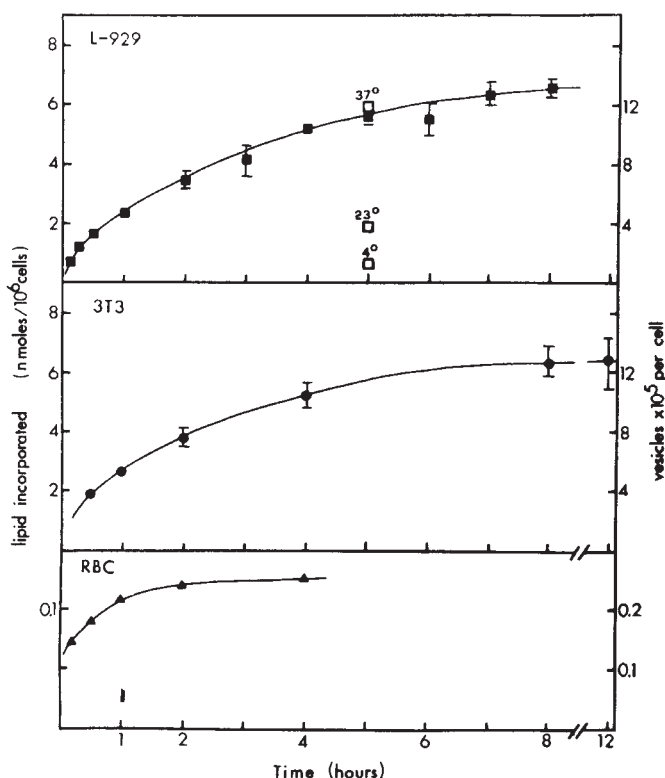


Fig. 1 Incorporation of unilamellar lipid vesicles by mouse 3T3 and L929 cells and human erythrocyte ghosts, at 37° C. Vesicles were prepared by dispersion and sonication of highly purified lipids in calcium and magnesium-free Hanks solution, pH 7.4, as before⁷ using 4 μ mol of phospholipid in 2 ml. Preparations were centrifuged at 100,000g for 30 min before use to remove contaminating multilamellar vesicles. Lipids were synthesised and purified as before⁷. Phosphatidylserine (PS) was from bovine brain and phosphatidylcholine (PC) from egg yolk. BALB/c mouse 3T3 cells and mouse L929 cells were cultured as before^{6,7}. Human erythrocyte ghosts were prepared from fresh blood by the method of Jung *et al.*⁸ which yields 'resealed' ghosts with a restored permeability barrier to small solutes. 3T3 and L929 cell populations were incubated with vesicles as before⁶. Erythrocyte ghost preparations were equilibrated in 50 mM NaCl, 2 mM histidine, 2 mM N-Tris (hydroxymethyl)-methyl-2-aminoethane sulfonic acid (TES) and 0.1 mM EDTA, pH 8.2, before incubation with vesicles. Unless stated otherwise, vesicle incorporation by cells was measured at 37° C by the uptake of ³H-dipalmitoyl-phosphatidylcholine (³H-DPPC) (specific activity 4 Ci mmol⁻¹) added in trace amounts during the formation of vesicles as described previously⁶. L929 cell monolayers and erythrocyte ghosts in suspension were incubated with vesicles composed of PS, PC and cholesterol (1 : 9 : 8 molar ratio and trace amounts of ³H-DPPC). 3T3 cell monolayers were incubated with vesicles containing stearylamine, PC and cholesterol (1 : 4 : 3 molar ratio and ¹⁴C-cholesterol as a tracer). 3T3 and L929 cells were exposed to an input of 50 nmol of phospholipid per 10⁶ cells (approximately 1×10^7 vesicle per cell). Erythrocyte ghosts were treated to an input dose of 1.3 nmol of phospholipid per 10⁶ cells. 3T3 and L929 cell monolayers were suspended by treatment with 0.2% trypsin and 0.2% EDTA in Hanks saline at intervals after incubation with vesicles and the amount of cell-associated radioactivity measured as before⁶. ■, L929 cells incubated at 37° C; □, L929 cells incubated at 37° C, 23° C and 4° C as designated in the figure; ●, 3T3 cells at 37° C; ▲, erythrocyte ghosts at 37° C.

Incorporation of lipid vesicles by mammalian cells provides a potential method for modifying cell behaviour

PHOSPHOLIPID vesicles (liposomes¹) have been used extensively as models for natural membranes^{1,2}. Multilamellar vesicles are incorporated by mammalian cells both *in vivo*^{3,4} and *in vitro*⁵. Sonication of such vesicles creates small (250-500 Å diameter)

vesicles)⁷ but the kinetics were different in each case. For 3T3 and L929 cell cultures, the maximum incorporation of vesicles shown in Fig. 1 represents between 2 and 5% of the vesicle population, while ghosts incorporated up to 10% of the vesicle population. Dye-exclusion measurements revealed no significant change in the viability of vesicle-treated 3T3 and L929 cells compared with untreated cells and their growth rate remained comparable with that of controls during successive subcultivations for 3 months. Figure 2 shows that vesicle uptake by 3T3 cells was linear over a 10,000-fold concentration range. Vesicle incorporation by L929 cells and erythrocyte ghosts was also generally similar. Chemical analysis of the lipid content of L929 cells and erythrocyte ghosts after a 6 h incubation with phosphatidylserine (PS)/phosphatidylcholine (PC) cholesterol (1:9:8 molar ratio) vesicles (conditions as in Fig. 1) revealed respectively a 20% and 15% increase in total phospholipid. Extraction of cell lipids in chloroform-methanol and thin-layer chromatography of the extracted lipids after incubation with PS/PC/cholesterol vesicles plus trace amounts of ³H-dipalmitoyl phosphatidylcholine (³H-DPPC) revealed that 70% of the incorporated ³H-DPPC label co-chromatographed with pure samples of PC, PS and phosphatidylethanolamine (PE). Tritium label was recovered from both plasma membrane and intracellular membrane fractions.

Uptake of unilamellar lipid vesicles by cultured cells was temperature-dependent (Fig. 1). Uptake of vesicles by cells was not significantly altered by inhibition of cellular energy metabolism by incubating cells for 30 min with sodium azide (2×10^{-4} M) or sodium iodoacetate (2×10^{-4} M) before addition of vesicles. Similarly, preincubation of cells with ouabain (10^{-3} M) for 1 h had no effect on vesicle incorporation, and pretreatment with $10 \mu\text{g ml}^{-1}$ polylysine (molecular weight 2,800) for 1 h to stimulate endocytosis⁹ also failed to alter the extent of vesicle uptake. Negatively charged (PS), neutral (PC) and positively-charged vesicles (20% stearylamine in PC) were incorporated equally well by cells. Comparable incorporation was also found using vesicles composed of lipids that were 'fluid' (10% PS in PC) or 'solid' (10% PS in distearoylphosphatidylcholine) at 37° C. Incorporation of equimolar amounts of cholesterol into 'fluid' (PS/PC) or 'solid' (PS/DPPC) vesicles produced no substantial change in cellular uptake of vesicles. In spite of these general similarities, 'fluid' lipid vesicles can be incorporated into cells by a different pathway than 'solid' vesicles (see below).

Vesicle components could be incorporated into cells by several non-exclusive mechanisms. Exchange diffusion of lipid molecules (cholesterol) between vesicles and natural membranes

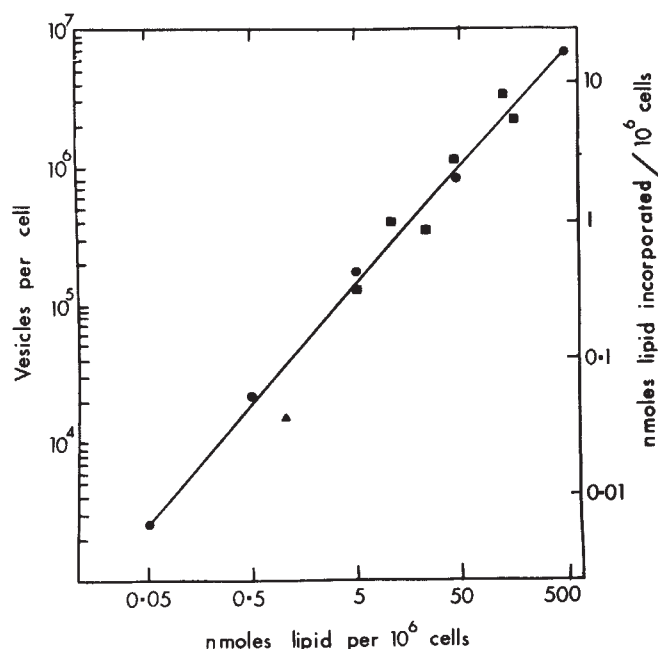


Fig. 2 Incorporation of unilamellar lipid vesicles by mouse 3T3 and L929 cells exposed to different concentrations of vesicles. 3T3 cells (●) were incubated for 4 h at 37° C with stearylamine, PC and cholesterol vesicles (1 : 4 : 3 molar ratio with ³H-DPPC as a tracer). Vesicle uptake was determined by measurement of cell-associated radioactivity as in Fig. 1. L929 cells (■) and erythrocyte ghosts (▲) were incubated for 4 h at 37° C with PS, PC and cholesterol vesicles (1:9:8 molar ratio with ³H-DPPC as a tracer).

has been documented^{10,11}. Alternatively, vesicles can be incorporated intact by cells either by fusing with the plasma membrane or by endocytosis. Finally, the recovery of 'markers' from vesicles in association with cells might merely reflect binding of vesicles to the cell surface without incorporation *per se*. Incubation of L929 or 3T3 cells with PS/PC/cholesterol vesicles containing trace amounts of ¹⁴C-cholesterol and ³H-DPPC 'markers' revealed that the ratio of ³H to ¹⁴C counts recovered in association with cells after incubation with vesicles was similar (1 : 1.8) to the ratio in the original vesicle population (1 : 1.9), suggesting incorporation of intact vesicles. The parallel uptake of ³H-DPPC and ¹⁴C-cholesterol suggests tha

Table 1 Effects of cyclic AMP trapped within lipid vesicles and free cyclic nucleotides on the growth of 3T3 cells

Treatment	Nucleotide concentration (M)	Cell growth as % control*	cyclic AMP incorporation % total†	cyclic AMP incorporation nmol per 10 ⁶ cells‡	Vesicle uptake % total§	Vesicle uptake nmol lipid per 10 ⁶ cells¶
'Fluid' vesicles	0	108.3	—	—	5.1	2.55
'Fluid' vesicles containing cyclic AMP	10^{-5}	25.6	4.9	0.98	5.4	2.70
'Solid' vesicles	0	105.4	—	—	4.6	2.31
'Solid' vesicles containing cyclic AMP	10^{-5}	97.2	4.2	0.84	4.9	2.45
Exogenous cyclic AMP	10^{-5}	94.0	0.4	0.08	—	—
Exogenous dibutylrlyl cyclic AMP	10^{-5}	70.3	NM	NM	—	—

Plastic Petri dishes (60 mm) were seeded with 3×10^5 3T3 cells and incubated at 37° C for 24 h. The culture medium was then replaced with medium containing unilamellar lipid vesicles, similar vesicles containing ³H-cyclic AMP or medium containing ³H-cyclic AMP plus 10^{-3} M theophylline or dibutylrlyl cyclic AMP plus 10^{-3} M theophylline, and the number of cells were counted at intervals. Control cultures were incubated in normal medium without vesicles or cyclic nucleotides. Vesicles containing cyclic AMP were prepared as described before⁶. The results represent mean values from three separate experiments. Fluid vesicles were stearylamine, PC and cholesterol (molar ratio 1 : 4 : 3); solid vesicles were stearylamine, DPPC and cholesterol (1 : 4 : 4, molar ratio). Both fluid and solid vesicles contained trace amounts of ¹⁴C-cholesterol.

*Calculated from cell counts on control and experimental cultures 6 d after inoculation.

†Cell-associated ³H-cyclic AMP measured after 24 h at 37° C as a percentage of the total ³H-cyclic AMP to which cells were exposed.

‡Calculated from values for cell-associated ³H-cyclic AMP after 24 h incubation at 37° C and known values for radioactivity per μmol ³H-cyclic AMP.

§Cell-associated ¹⁴C-cholesterol measured after 24 h at 37° C and expressed as a percentage of the radioactivity in the original vesicle population.

¶Calculated from the cellular incorporation of ¹⁴C-cholesterol after 24 h of incubation with the vesicles and the specific activity of the cholesterol in the vesicles.

NM, not measured.

molecular exchange between cell membranes and vesicles is not the major mechanism for uptake of vesicle components since cholesterol would exchange at a faster rate¹⁰. The lack of effect of inhibitors of energy metabolism on vesicle uptake (see above) suggests that classical endocytosis is probably not involved, though incorporation of material with molecular weights lower than 2×10^5 by metabolically-independent endocytotic mechanisms has been described¹².

We have also studied the cellular uptake of vesicles (stearylamine/PC/cholesterol; 1 : 4 : 3 molar ratio) containing ³H-cyclic adenosine 3' : 5' monophosphate (³H-cyclic AMP) trapped within the internal aqueous space⁶. Incubation of 3T3 cells with such vesicles resulted in significant uptake of both ¹⁴C-cholesterol and ³H-cyclic AMP molecules from the vesicles (Table 1). Vesicle-incorporated cyclic AMP produced significantly greater inhibition of cell growth than equivalent concentrations of exogenous cyclic AMP or dibutyl cyclic AMP plus theophylline (Table 1). Since the growth inhibition by exogenous nucleotides was similar to that reported for free nucleotides¹³, our results indicate that vesicles offer an efficient carrier vehicle for increasing the uptake of biologically important molecules into cells. The ratio of ³H to ¹⁴C counts recovered from vesicle-treated cells was similar to that of the original vesicle population, supporting the earlier suggestion that intact vesicles are incorporated.

The alteration of cell growth by cyclic AMP-containing vesicles suggests that active cyclic AMP is released from vesicles into the cytoplasm. The simplest, but not the only possible interpretation for this presumed cytoplasmic distribution of cyclic AMP, is that some vesicles may fuse with the plasma membrane, introducing their contents directly into the cytoplasm. Using the same experimental protocol, ³H-cyclic AMP trapped within vesicles prepared from 'solid' lipids (stearylamine/DPPC/cholesterol) did not induce significant alteration in cell growth in spite of significant vesicle uptake (Table 1). This contrasting effect between 'fluid' and 'solid' vesicles could reflect differences in the 'leakiness' of the two types of vesicles for the trapped cyclic AMP. Alternatively, the vesicles may differ in their ability to fuse with the cell surface. 'Solid' lipid vesicles are unable to fuse cells⁷ and have a very limited capacity

to fuse with each other compared with 'fluid' vesicles¹⁴. 'Solid' vesicles containing cyclic AMP might not, therefore, be able to fuse with the plasma membrane and may be incorporated primarily by endocytosis, introducing their contents instead into the lysosomal apparatus. This would reduce the likelihood of cyclic AMP reaching the cytoplasm in an active form, since lysosomes contain several phosphodiesterases¹⁵.

The incorporation of lipid molecules from 'fluid' vesicles into the plasma membrane, presumably by fusion, is further suggested by the recovery of radiolabelled lipid in the plasma membrane fraction of fractionated cells (see above) and also by changes in the lectin agglutinability, and membrane transport properties of vesicle-treated cells (our work in preparation). Figure 3 shows a freeze-fractured erythrocyte ghost after incubation with multilamellar PS/PC vesicles. The 'bare patches' on the ghost were found in 5% of the treated cells, but were not found on several hundred fields of untreated controls. The dimensions and surface topography of the 'patches' are very similar to vesicles and it is tempting to interpret that these areas represent sites where vesicles have fused with the plasma membrane. Also, the density of intramembranous particles on vesicle-treated cells not showing 'patches' was significantly lower than in untreated cells (our work in preparation), which suggests that the cell surface area had increased.

Our results indicate that mammalian cells can incorporate large numbers of lipid vesicles without cytotoxic effects. Fusion of vesicles with the plasma membrane may represent an important pathway for vesicle incorporation, though endocytosis, surface adsorption and molecular exchange between vesicles and cell membranes may also be occurring simultaneously. Although the precise contribution of different pathways in the incorporation of vesicles into cells remains to be elucidated, the present results, together with other recent studies, indicate that lipid vesicles offer a potentially useful tool for modifying cell behaviour by introducing new material into the plasma membrane (ref. 16 and our work in preparation), and by the delivery of biologically active molecules and drugs into the cell³⁻⁶. Independent data on vesicle uptake by mammalian cells are given by Pagano *et al.*¹⁸ in the next communication.

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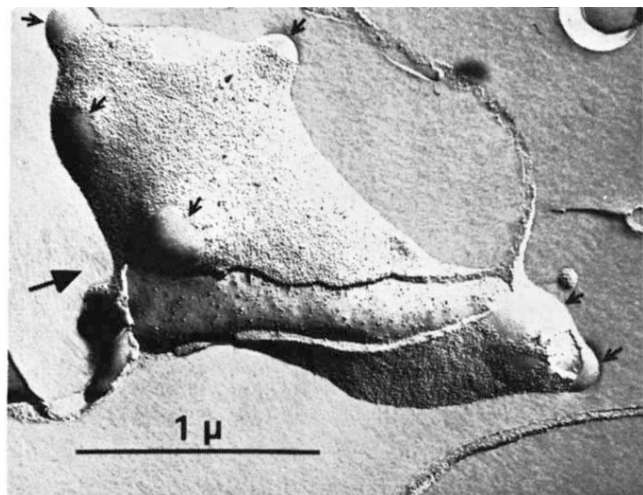


Fig. 3 Electron micrograph of freeze-fractured human erythrocyte ghost after incubation with multilamellar lipid vesicles, showing areas (small arrows) devoid of intramembranous particles. Vesicles (PS/PC, 1 : 9 molar ratio) were dispersed by mechanical (vortex) agitation in 50 mM NaCl, pH 8.2. Incubation with ghosts was performed in the same buffer with 1.3 nmol phospholipid per 10^8 cells, for 30 min at 37° C. The cells were spun down at room temperature and resuspended in 30% (v/v) glycerol, frozen in Freon 22, fractured at -115° C in a Balzers BA360 apparatus and shadowed with platinum-carbon as before¹⁷. Large arrow indicates direction of shadowing.

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Interaction of phospholipid vesicles with cultured mammalian cells

To delineate some of the relationships between membrane composition and the functional states of mammalian cells, we are exploring the possibility of producing controlled modifications in the plasma membrane of cultured cells, using artificially generated lipid vesicles¹⁻³. Although other groups have reported the use of lipid dispersions for producing cellular modifications⁴⁻¹⁰, the underlying molecular mechanisms by which these effects are produced have not been determined. We present here preliminary findings on the mechanism of interaction of chemically and physically well-defined phospholipid vesicles with cultured mammalian cells.

Typical data obtained when Chinese hamster V79 cells were incubated with vesicles produced from radiolabelled egg yolk lecithin are given in Fig. 1. Unilamellar (F-II) vesicles were more efficient in the transfer of exogenous phospholipid to the cells than multilamellar (F-I) vesicles. In both cases a considerable amount of phospholipid became associated with the cells in a relatively short time. The amount of lipid taken up depended on the physical state of the lipids comprising the vesicles, being substantially greater below the gel-liquid crystalline phase transition temperature. Between 20% and 30% of the labelled phospholipid taken up by vesicle-treated cells appeared in their plasma membranes. This lipid was not degraded, but remained as lecithin. The remaining radioactivity was distributed into intracellular fractions in which considerable degradation was observed. No effect on cell viability could be detected for short periods of incubation (up to 6 h), and normal cell growth resumed when the vesicle suspension was replaced by fresh medium. Prolonged incubation of sparse cultures ($\sim 10^3$ cells cm^{-2}), however, resulted in considerable cell death as determined by dye exclusion tests. This deleterious effect of lipid vesicle treatment agrees with our observation that sparse cultures take up about an order of magnitude more phospholipid than those approaching confluency.

Figure 2a shows a transmission electron micrograph obtained from a thin section through a preparation of V79 cells incubated with a suspension of F-I vesicles. A large vesicle, about 7,000 Å in diameter, with the characteristic multilamellar appearance of this vesicle fraction, is seen in direct contact with the plasma membrane of the cell. Figure 2b shows a preparation of unilamellar vesicles, about 500 Å in diameter, incubated with V79 cells pretreated with cationised ferritin¹² (0.5 mg ml^{-1} for 5 min at 25°C). There are numerous vesicles, both attached to the surface membrane and apparently fused with the cell. Although there were no essential differences in the amount of phospholipid taken up in the presence or absence of cationised ferritin, the fate of the applied unilamellar vesicle suspension could not be traced by electron microscopy without this marker.

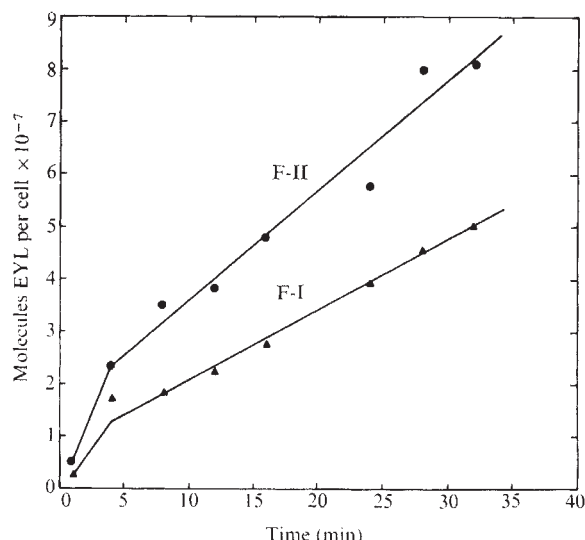


Fig. 1 Uptake data obtained for V79 cells incubated with egg yolk lecithin (EYL) vesicles at 25°C. Vesicles were prepared by ultrasonic irradiation of radiolabelled EYL in Gey's balanced salt solution¹¹. The radiolabelled lipid was prepared by adding a trace amount of ~ 5 Ci mmol^{-1} ^3H -distearoyl lecithin to EYL to give a product with a final specific activity of 1 mCi mmol^{-1} EYL. The lipid dispersion was fractionated by gel permeation chromatography³ into multilamellar (F-I) and unilamellar (F-II) fractions, and diluted in each case to a concentration of 0.09 mM lipid phosphorus. In a typical experiment, a culture plate containing $\sim 10^7$ cells was washed in Gey's, followed by the addition of the labelled vesicle suspension. After incubation the plate was washed thoroughly, the cells briefly trypsinised, and the resulting cell suspension repeatedly centrifuged and resuspended in Gey's. The radioactivity in the final pellet was determined, and the total number of phospholipid molecules taken up per cell was calculated. Error is $\pm 5\%$.

The uptake of phospholipid from either unilamellar or multilamellar vesicles by cells was insensitive to inhibitors of energy metabolism (30 mM NaN_3 ; 0.1 mM dinitrophenol). Cells pretreated with 2% glutaraldehyde (2 h at 4°C) before uptake showed about a 25% reduction in the amount of phospholipid taken up after 1 h of incubation. These observations strongly suggest that the incorporation of exogenous phospholipid from vesicles by the cultured cell system examined here is not energy dependent. Accordingly, in considering the possible mechanisms of uptake, we rule out active processes such as endocytosis, and consider only the following: (i) vesicle-cell fusion; (ii) phospholipid exchange, similar to that described for *in vitro*^{13,14} systems in which phospholipid molecules from the vesicle suspension and plasma membrane of the cell are interchanged and (iii) adsorption, in which the exogenous

Table 1 Uptake by Chinese hamster V79 cells of ^{14}C -dioleoyl lecithin vesicles containing ^3H -inulin*

	c.p.m. found in vesicle-treated cells†		Ratio (^{14}C c.p.m./ ^3H c.p.m.)	
	^{14}C -dioleoyl lecithin	^3H -inulin	Observed	Expected
Multilamellar vesicles				
2°C	126	~ 5	≥ 25	0.41
37°C	370	108	3.4	0.41
Unilamellar vesicles				
2°C	220	42	5.2	0.44
37°C	800	1,000	0.8	0.44

* Doubly labelled vesicles were prepared by cosonication of ^{14}C -dioleoyl lecithin and ^3H -inulin. The vesicle fractions containing the trapped marker were subsequently separated from free inulin by chromatography on Sepharose 4B. No leakage of the trapped marker could be detected during this experiment.

† Protocol as in Fig. 1; 1 h incubation.

phospholipid from the applied vesicles is adsorbed to the cell surface, resulting in a net transfer of lipid to the cell.

If fusion is involved in the interaction of phospholipid vesicles with cultured cells, then the aqueous contents of such vesicles should be released into the cytoplasm of vesicle-treated cells after each fusion event. To test this possibility, vesicles comprised of ^{14}C -dioleoyl lecithin and containing ^3H -inulin as a marker for the internal aqueous volume were used in an uptake experiment (Table 1). If all the phospholipid enters the cell by fusion, the ratio of phospholipid to inulin radioactivity found associated with vesicle-treated cells should be that of the applied vesicle suspension. Table 1 shows the amounts of ^{14}C -dioleoyl lecithin and ^3H -inulin found associated with cells treated with either multilamellar or unilamellar vesicles together with the observed and expected ratios of c.p.m. of ^{14}C to tritium. The observed ratios were calculated from the ^{14}C -lecithin and ^3H -inulin taken up by the cells for each treatment, whereas the expected values were derived directly from the measured ratios of the applied vesicle fractions. In each case the amount of the trapped marker, ^3H -inulin, which became cell-associated was not proportional to the

The use of lipid vesicles bearing a net charge to entrap soluble markers of opposite charge raises the question of whether such markers are bound or associated with the wall of the lipid vesicle. If such an interaction exists, the bound marker might enter the cell by some internalisation mechanism and not by fusion. Second, the marker once associated with the cell must be demonstrated to be free within the cytoplasm of the cell and not compartmentalised either in the lysosomal apparatus or in some other membrane-bound vesicular body. Although we believe that the first condition has been fulfilled in our studies, we have no sufficient proof that the second criterion has been met. Thus we can only suggest vesicle-cell fusion as an explanation for our observations. Nevertheless, these studies should provide a useful basis for achieving modification of the surface properties of mammalian cells.

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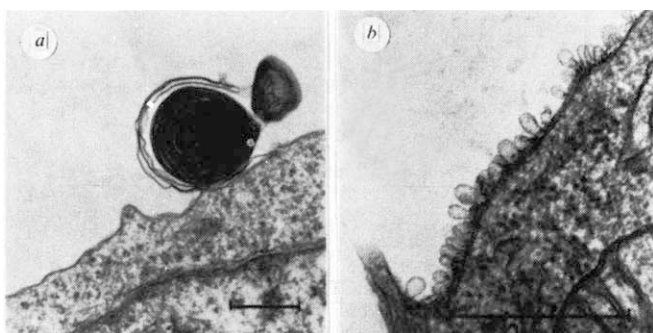


Fig. 2 Transmission electron micrographs of V79 cells incubated for 1 h at 37° C (a) with multilamellar vesicles generated from EYL, and (b) with unilamellar vesicles generated from dioleoyl lecithin. In (b), cells were pretreated with cationised ferritin (see text). Cells were pre-fixed in 0.1 M cacodylate buffer (pH 7.2) containing 2% glutaraldehyde, fixed in 1% OsO₄, stained in uranyl acetate and embedded in Epon. Thin sections were stained with lead citrate. The bar represents 0.5 μm .

amount of phospholipid taken up. For example, for multilamellar vesicles at 37° C, the observed ratio of phospholipid c.p.m. to inulin c.p.m. was 3.4, compared with an expected value of 0.41. If the trapped marker can become associated with the cell only as the result of fusion, we calculate that at 2° C, only small amounts of the observed phospholipid uptake (<2% for multilamellar and ~10% for unilamellar vesicles) can be accounted for by fusion, whereas at 37° C, more significant fractions of the lipid uptake (~10% for multilamellar and ~50% for unilamellar vesicles) could enter the cells by such a mechanism. The remaining lipid would have to be taken up by some other pathway such as phospholipid exchange or adsorption. Experiments are in progress to test these possibilities. Similar studies of cells pretreated with glutaraldehyde demonstrated that the association of the trapped marker with vesicle-treated cells was not significantly inhibited, thus confirming the supposition that vesicle uptake is not an active process.

To demonstrate unequivocally that a fusion process is involved in the overall mechanism of uptake of unilamellar vesicles by cultured cells, we require in addition to the uptake of phospholipid and trapped marker, that the following criteria be met. First, the trapped marker must be truly soluble in the internal aqueous space of the vesicle.

Yeast mitochondrial RNA does not contain poly(A)

POLY(A) is a universal constituent of eukaryotic messenger RNAs with the exception of histone mRNA¹. Recently Penman² and Attardi³ have demonstrated that poly(A) was also present in mitochondrial RNA from animal cells. This finding was of interest to us for two reasons. First, it suggested a simple means of isolating mitochondrial mRNAs and, second, it suggested that the similarity between prokaryotes and mitochondria might not be as close as previously suggested⁴. We therefore looked for poly(A)-containing RNA (poly(A)-RNA) in the simple eukaryote *Saccharomyces carlsbergensis*.

Intact yeast mitochondria are difficult to isolate quickly from whole cells. Since rapid isolation should be a prerequisite for the characterisation of RNA pulse labelled for short periods, we preferred to pulse label isolated protoplasts. Using this system McLaughlin *et al.*⁵ have detected considerable amounts of poly(A)-RNA in yeast polysomal RNA. Details of our protoplast preparation are described elsewhere (G.S.P.G., and R. O. Poyton, unpublished).

Yeast protoplasts were pulse labelled for 30 min with ^3H -adenine, then quickly cooled and lysed by osmotic shock. After the removal of nuclei and cell debris, mitochondrial and post-mitochondrial fractions were prepared⁶ and RNA extracted from these by the hot-phenol-chloroform method of Penman⁷. After extraction the RNA was precipitated with two volumes of ethanol and stored at -20°C overnight. The recoveries of both ^3H -labelled RNA and added ^{32}P -labelled poly(A), as determined by acid-insoluble radioactivity, were always greater than 90%.

To test whether poly(A)-RNA was present, aliquots (approximately 20,000 c.p.m.) of the mitochondrial and cell-sap RNA were fractionated on oligo(dT) cellulose columns as described by Faust *et al.*⁸. The results of such a fractionation are given in Fig. 1. It can be seen that, whereas 30% of the cell-sap RNA is retained by the column and elutes at low salt concentrations (Fig. 1b), less than 0.6% of such material is present in the mitochondrial RNA (Fig. 1a). The binding of the mitochondrial RNA was unaffected by previous denaturation (2 min, 100°C in 0.5 mM EDTA, pH 7.5). As an internal control ^{32}P -labelled poly(A) was added and indeed eluted exclusively at low salt concentrations. To exclude the possibility that the cell-sap poly(A)-RNA was bound by a nonspecific process, competing poly(U) was added to a second aliquot of this RNA plus internal ^{32}P -labelled poly(A). As expected, both the ^3H and ^{32}P radioactivity was no longer retained by the oligo(dT) cellulose (Fig. 1c), demonstrating that the cell-sap RNA was indeed bound by its poly(A) moiety. Since the recovery from our oligo(dT) cellulose columns was 95–110%, we conclude that mitochondrial RNA pulse-labelled for 30 min, does not contain a poly(A) sequence of sufficient length to bind it to the oligo(dT) cellulose column. Identical results were obtained using chromatography on poly(U) Sepharose.

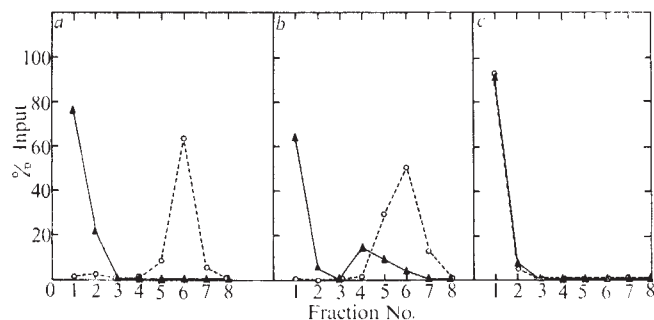


Fig. 1 Fractionation of pulse-labelled RNA on oligo(dT) cellulose columns. 4 g of yeast (*S. carlsbergensis* NCYC 74) protoplasts were labelled for 30 min at 28°C with ^3H -adenine (17 mCi l^{-1}) in 200 ml growth medium, supplemented with 1.0 M sorbitol; cell-sap and mitochondrial RNA was prepared as described in the text; aliquots (20,000 c.p.m.) in 0.5 M KCl, 0.01 M Tris, pH 7.5, were mixed with ^{32}P -labelled poly(A) (10,000 c.p.m.), prepared as described¹⁴, and applied to the oligo(dT) cellulose column. The flow-through material was passed again over the column. Fraction 1: final flow-through; fraction 2, elution with 0.5 M KCl, 0.01 M Tris; fraction 3, 0.25 M KCl, 0.01 M Tris; fraction 4, 0.1 M KCl, 0.01 M Tris; fraction 5, 0.05 M KCl, 0.01 M Tris; fraction 6, 0.01 M Tris; fraction 7, 95% formamide, 0.01 M Tris; fraction 8, 0.1 M NaOH. The fractions were then precipitated with 5% trichloroacetic acid after the addition of 100 μg bovine serum albumin. The precipitates were collected on Whatman glass fibre filters (GF-81) and counted. Δ , 30 min ^3H -adenine pulse-labelled RNA; $\circ$ — $\circ$, ^{32}P -poly(A). a, Mitochondrial RNA; b, cell-sap RNA; c, cell-sap RNA + poly(U).

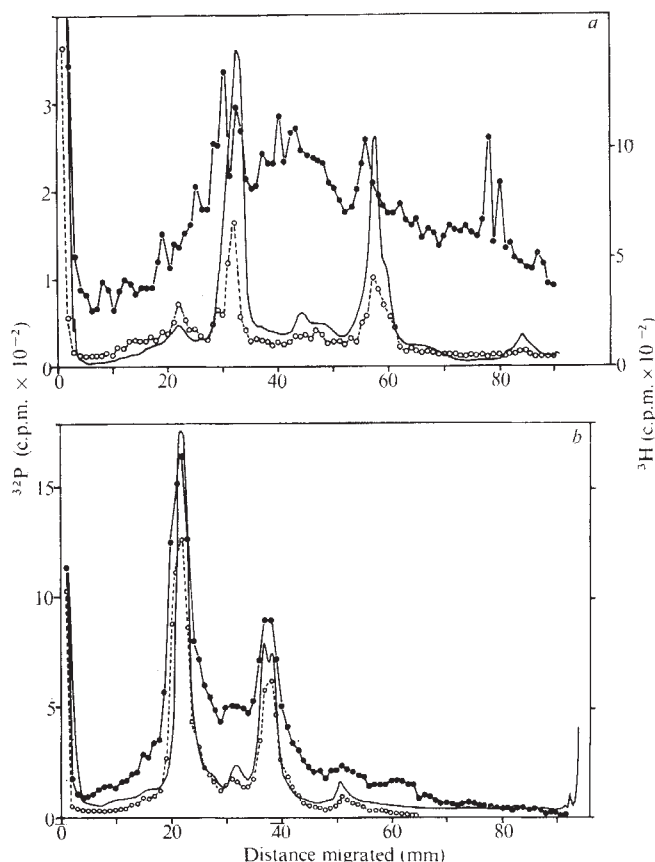


Fig. 2 Polyacrylamide gel analysis of pulse-labelled mitochondrial RNA from yeast. Electrophoresis conditions: 2.4% acrylamide gels using the buffer system of Peacock and Dingman¹⁵. After scanning at 260 nm (—), gels were frozen at -70°C , sliced, extracted and counted¹⁶. $\circ$, ^3H -adenine long-labelled mitochondrial RNA added as internal marker during electrophoresis; $\bullet$, ^{32}P pulse profile. a, 4 min pulse; b, 15 min pulse.

To minimise the chance that poly(A)-RNA was missed in our experiments because of degradation during the isolation of the mitochondria, the cell-sap fraction was maintained in ice for the same length of time used for the mitochondrial purification (2 h). The finding of 30% of the cell-sap RNA as poly(A)-RNA renders this possibility unlikely.

We considered it possible that the *in vivo* turnover rate of poly(A)-mitochondrial RNA was so rapid that very little could be detected in a 30-min pulse in contrast to the cell-sap system. We therefore prepared mitochondrial RNA pulse-labelled for 4, 15 and 30 min, respectively. Polyacrylamide gel electrophoresis of two of these preparations is shown in Fig. 2. The 4 min pulse-labelled preparation clearly contains much heterogeneous RNA, indicating that processing and maturation of at least rRNA, has not occurred. This is clearly evident in the 15-min pulse-labelled material. The presence of intact endogenous rRNAs in these preparations, however, excludes trivial explanations for the heterogeneous RNA such as degradation. The 4 min and 30 min pulse preparations were also applied to the oligo(dT) cellulose column (Table 1). Again less than 0.5% of the ^{32}P -labelled RNA was retained by the column in contrast to the complete retention of the internal ^3H -labelled poly(A) control. Total RNA from protoplasts pulse-labelled for 5 min with ^3H -adenine contained 20% poly(A)-RNA. Moreover, we were able to show considerable amounts of poly(A)-RNA in the mitochondrial RNA from chick fibroblasts (Table 1). Finally, we have analysed RNA synthesised by isolated yeast mitochondria *in vitro* for poly(A) content. Again less than 0.4% poly(A)-RNA could be detected.

As explanation of these results, two possibilities remain: either there is no poly(A)-mitochondrial RNA in yeast or the

Table 1 % Poly(A)-RNA in pulse-labelled mitochondrial RNA

Labelling condition	Organism	Radio-active precursor	Pulse time (min)	Input applied to oligo(dT) cellulose (c.p.m.)	% RNA retained
<i>In vivo</i>	Yeast	³² P-P _i	4	10,500	0.5
<i>In vivo</i>	Yeast	³² P-P _i	30	42,000	0.5
<i>In vivo</i>	Yeast	³ H-adenine	30	4,840	0.6
<i>In vitro</i>	Yeast	³ H-UTP	20	5,100	0.4
<i>In vivo</i>	Chick	³ H-uridine	15	16,100	34.4

Yeast protoplasts (5–15 g, 20 g l⁻¹) were pulse-labelled with either ³²P-orthophosphate (25–40 mCi l⁻¹) or ³H-adenine (4 mCi l⁻¹) and mitochondrial RNA isolated as described in Fig. 1. To measure *in vitro* RNA synthesis, isolated mitochondria were incubated as described¹¹, except that the medium was supplemented with 50 μM ³H-UTP (specific activity 10⁶ c.p.m. nmol⁻¹). Chick fibroblasts were grown and pulse-labelled with ³H-uridine as described¹² and mitochondria were purified as described by Borst *et al.*¹³. RNA was prepared and oligo(dT) cellulose chromatography performed as described in Fig. 1. The % retained is defined as the counts eluting in fractions 4–8.

poly(A) region is so short that it cannot be retained by the oligo(dT) cellulose and poly(U) Sepharose columns. Preliminary experiments showed that while polyacrylamide gel electrophoresis was capable of resolving a 50 nucleotide poly(A) segment⁵ derived from cell-sap RNA by ribonuclease digestion⁸, no such component was present in mitochondrial RNA. Under these conditions, however, a poly(A) tract of less than 30 nucleotides would not have been detectable above the high background of completely digested RNA peaking ahead of the bromophenol blue marker. Since Jacobson *et al.*⁹ showed that a poly(A) segment of 25 was sufficient to bind *Dictyostelium* nuclear RNA to poly(U) Sepharose, we presume that if mitochondrial RNA contains a poly(A) segment, it must be considerably shorter than this. Such a poly(A) region differs so much from its homologue in the animal cell, that its possible existence becomes somewhat a semantic problem.

To us the most likely interpretation of our data is that mitochondria from yeast contain no poly(A)-RNA in contrast to animal mitochondria and in common with the RNA of prokaryotes and histone mRNA. Such a result is consistent with a prokaryotic origin for mitochondria and tempts us to suggest that poly(A)-mRNA in animal mitochondria is the result of adaption of a 'prokaryotic' genetic system to the demands of life in the higher eukaryote which occurred parallel to the loss of genetic information in the animal mitochondrial DNA (the potential coding capacity of yeast mitochondrial DNA is two to five times that of animals).

Finally, our results are in conflict with those of Cooper and Avers¹⁰, who reported poly(A)-mitochondrial RNA in yeast mitochondria. We attribute their results to contamination of the mitochondrial preparations with intact protoplasts.

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Large plasmid in *Agrobacterium tumefaciens* essential for crown gall-inducing ability

THE gram-negative bacterium *Agrobacterium tumefaciens* induces crown gall tumours in many, mostly dicotyledonous, plants. Zaenen *et al.*¹ demonstrated the presence of one or more large plasmids in a number of crown gall-inducing *Agrobacterium* strains belonging to seven different *Agrobacterium* groups. They were not able to find such plasmids in eight non-pathogenic *Agrobacterium* strains belonging to four of the same groups^{2,3}. They therefore formulated the hypothesis that the genetic information for the tumour-inducing principle⁴ in crown gall-inducing *Agrobacterium* strains is carried by one or several large plasmids.

Here we determine whether the large plasmids present in crown gall-inducing *Agrobacteria* are essential to the tumour-inducing capacity of such strains. We show that, in a non-tumorigenic derivative of a crown gall-inducing strain, the stable conversion to non-tumorigenicity is correlated with a concomitant loss of the large plasmid present in the parental strain. Second, we show for one tumour-inducing *Agrobacterium* strain that curing this strain of its plasmid in all cases resulted in the stable conversion of the cured strains to non-tumorigenicity, the correlation between the loss of the plasmid and the loss of the tumour-inducing plasmid (TIP) being 100%.

The crown gall-inducing *Agrobacterium tumefaciens* strain IIB and two of its derivatives, the tumorigenic strain IIBV7 and the non-tumorigenic strain IIBNV6, both isolated from strain IIB as single colonies, were compared (Braun, A. C., unpublished). Using ultracentrifugation on neutral sucrose gradients and caesium chloride–ethidium bromide equilibrium density centrifugation in the conditions described by Zaenen *et al.*¹, we demonstrated the presence of a large plasmid in *A. tumefaciens* strains IIB and IIBV7. Fractions containing the plasmid peaks were examined under the electron microscope and large double-stranded DNA supercoiled molecules were photographed. Only the non-tumorigenic strain IIBNV6 did not contain a large plasmid when examined repeatedly.

Hamilton *et al.*⁵ reported that the crown gall-inducing strain C58 can very reproducibly be converted to a non-tumorigenic

Table 1 Bacterial colonies which can induce crown gall tumours also harbour large plasmids

No. generations of growth at 37° C	No. colonies tested for pathogenicity	No. crown-gall forming colonies	No. TIP ⁺ colonies tested for the presence of the plasmid	No. plasmid positive colonies	No. TIP ⁻ colonies tested for the presence of plasmid	No. plasmid-negative colonies
0	5	5	—	—	—	—
2	5	5	—	—	—	—
4	5	5	2	2	—	—
10	10	8	8	8	2	2
19	15	3	3	3	8	8
38	15	0	—	—	1	1

strain by simple growth at 37° C. The resulting non-tumour-inducing bacteria do not revert to tumorigenicity. We found that derivatives of *A. tumefaciens* strain B6 could neither be cured of their plasmid nor converted to non-tumorigenicity by growth at 37° C. For further study we therefore decided to concentrate on strain C58. By neutral sucrose gradient and caesium chloride-ethidium bromide centrifugation and subsequent electron microscopy, strain C58 was shown to harbour a large plasmid. Length contour measurements were carried out on open circular molecules. Analysis of the results showed an unimodal distribution, the mean contour length being $61.8 \pm 1.1 \mu\text{m}$ (s.d.).

To obtain independently-isolated non-tumorigenic derivatives and to check the reproducibility of the conversion to non-tumorigenicity, strain C58 was streaked out for single colonies on a YEB agar plate¹ and incubated for 5 d at 37° C. Six of the resulting colonies were separately collected, diluted and plated for single colonies on six different YEB agar plates at 28° C. Twenty-five colonies from each plate were tested for tumour induction on wounded seeds of *Pisum sativum*⁶. All 150 colonies tested proved to be non-tumorigenic. A total of 12 non-tumorigenic colonies (two from each plate) were cultured at 28° C and their DNA analysed on neutral sucrose gradients¹. These non-tumorigenic bacteria were found to have lost the large plasmid present in the parental strain C58.

These results provide strong support for the hypothesis that the genetic information for the tumour-inducing principle in crown gall-inducing *Agrobacterium* strains is carried by one or more plasmids. The loss of tumour-inducing ability and loss of the plasmid, however, may be two unrelated events both brought about by growth at 37° C. If this were the case one would expect that in conditions where neither the curing of the plasmid nor the conversion to non-tumorigenicity would have occurred for all the population, one would find some bacteria for which only one of these events would have taken place. In order to realise such conditions we studied the kinetics of appearance of non-tumorigenic and of plasmid free bacteria in a strain C58 culture grown at 37° C.

An exponential culture of strain C58 grown in YEB medium at 28° C was diluted in fresh YEB medium to about 10^4 cells ml^{-1} and incubated at 37° C. When a titre of 10^9 cells ml^{-1} was reached, the culture was diluted to a titre of 10^4 cells ml^{-1} into fresh medium and further incubated at 37° C. This procedure was repeated several times. At different intervals samples were taken, the bacteria were diluted and plated for single colonies on YEB plates at 28° C. From each sample, several single colonies were tested on pea seedlings for tumour induction.

Several tumorigenic and non-tumorigenic colonies derived from different samples were tested for the presence of a large plasmid. To be able to handle more colonies when searching for the presence of plasmids in *Agrobacteria* we modified the neutral sucrose gradient technique of Zaenen *et al.*¹ by adding $1 \mu\text{g ml}^{-1}$ ethidium bromide (final concentration) to our sucrose gradients. Plasmid bands can be seen when the nitrocellulose centrifuge tubes are illuminated with long wave ultraviolet light. This eliminates the time consuming radioactive labelling, fractionating and liquid scintillation counting procedures.

All bacterial colonies that could induce crown gall tumours also harboured the large plasmid (Table 1), whereas all bacteria which had lost the ability to induce tumours had also lost the

plasmid. In all experiments special care was taken to make certain that we were not dealing with contaminants. This could easily be achieved by the use of a set of virulent *Agrobacterium* phages towards which strain C58 shows a characteristic sensitivity pattern.

As in all our experiments the correlation between loss of tumour-inducing capacity and loss of a large plasmid was found to be a 100%, we conclude that the presence of this plasmid is essential to the tumour-inducing ability of these strains. The most likely explanation for this would be that the plasmid in itself carries the genetic information for the tumour-inducing principle.

Such a conclusion, however, can only be reached if one can demonstrate induction of crown gall tumours with purified plasmid DNA. Our observations point to the possibility that bacterial plasmids could be oncogenic. Further work will be required to decide whether the role of this plasmid in tumour induction is directly due to the fact that it carries the genes of the tumour-inducing principle or merely because it somehow promotes the transfer of the TIP from the bacterium to the plant cell.

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Multiple divergent copies of endogenous C-type virogenes in mammalian cells

It is characteristic of endogenous C-type viruses that all individual animals of a species (as well as all tissues of each animal)

contain, in the chromosomal DNA, nucleic acid sequences that can code for the production of complete C-type viral particles. It is postulated that these sequences (C-type virogenes) are transmitted from parent to progeny with the other cellular genes¹. Nucleic acid hybridisation, using either single-stranded or double-stranded ³H-DNA transcripts or labelled 70S RNA as viral probes, has revealed C-type viral nucleic acid sequences in DNA from uninfected tissues of avian²⁻⁵, murine^{6,7}, porcine⁸, feline⁹⁻¹² and Old World monkey species¹³.

We have now examined the reiteration frequency of mouse, rat, pig, cat and baboon viral nucleic acid sequences in tissues and cell cultures of the species of origin of these viruses and in exogenously infected cells of heterologous species. While DNA from each species contains multiple copies of endogenous viro-gene sequences per haploid cellular genome (5–15), cell lines of heterologous species infected with and producing high titres of these C-type viruses contain fewer copies (one or two per haploid genome). The thermal stability of DNA:DNA hybrids demonstrates that the endogenous virogenes are composed of families of genes with related but not identical nucleic acid sequences.

We examined a xenotropic (S-tropic) virus isolated from a murine BALB/3T3 cell line¹⁴; the RD114/CCC group of endogenous cat viruses^{15,16}; a C-type virus isolated from normal baboon tissue¹⁷; a porcine C-type virus spontaneously released by the pig kidney cell line, PK(15)⁸; and a rat C-type virus produced by a rat thymus cell line¹⁸.

The reassociation kinetics shown in Fig. 1 can be used to estimate relative gene frequencies by determination of the half C_0t values (the midpoint of the renaturation curve)¹⁹. Figure 1a shows that the half C_0t value for the hybridisation of the murine C-type viral ³H-DNA probe to normal BALB/c strain mouse liver or to BALB/3T3 cell line DNA is 1.1×10^2 – 1.2×10^2 mol $\times$ s l⁻¹; whereas the half C_0t value for hybridisation with the DNA of either a canine or rabbit cell line infected with this virus is 1.5×10^3 . The half C_0t value for the self-annealing of unique sequence rabbit and dog (Fig. 1), as well as mouse, cat, baboon and human cellular DNA has varied between 1×10^3 and 2×10^3 mol $\times$ s l⁻¹ (ref. 13). There is, therefore, only one or at most two copies of mouse C-type virus in the heterologous infected dog or rabbit cell lines, and there are approximately ten times this number of copies in the mouse cell line or in normal mouse tissue.

Sequences homologous to probes prepared from the endogenous baboon virus can be found in the DNA of various tissues of normal baboons¹³; as shown in Fig. 1b, the half C_0t value for this hybridisation is 1.0×10^2 – 1.4×10^2 . In contrast, a cloned cell line infected with the baboon virus contains only one copy per haploid cell DNA (half C_0t value is 1.0×10^3).

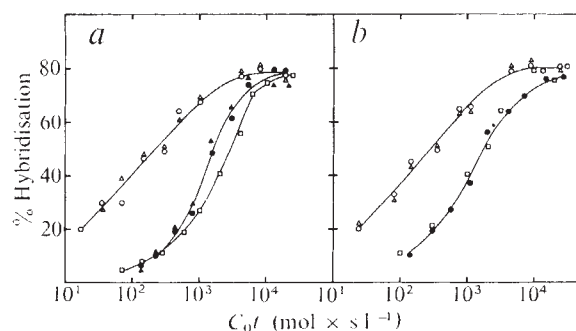


Fig. 1 Hybridisation of murine and baboon C-type viral ³H-DNA probes to DNA extracted from normal murine and baboon tissues and cell lines, and from cells from heterologous species infected with these viruses. The ³H-thymidine-labelled DNA probes were synthesised from detergent-disrupted C-type virus in the presence of actinomycin D²⁷. The specific activity of the ³H-DNA was 1.5×10^7 c.p.m. μ g⁻¹. The ³H-DNA probes contained 50–75% of their respective 70S viral RNA sequences at a ³H-DNA:³²P-viral RNA molar ratio of 1.5¹³, indicating that they contained most of the sequences present in viral RNA and that these sequences were in proportions similar to their content in 70S RNA. Cellular DNA was extracted from tissues and cell lines¹³. All DNAs were treated sonically to yield a mean size of 6–8S (the size of the ³H-DNA probes) as determined by centrifugation on alkaline sucrose gradients¹³. DNA:DNA hybridisations were incubated at 65° C in reaction mixtures containing 0.01 M Tris, pH 7.4, 0.75 M NaCl, 2×10^{-3} M EDTA, 0.05% Sodium dodecyl sulphate, 10,000–20,000 c.p.m. ml⁻¹ of ³H-DNA and 1–3 mg of cellular DNA per ml. Hybridisations were started by heating the mixtures to 98° C for 10 min, cooling on ice to 4° C, and incubating at 65° C. At various times (from 15 min to 96 h) 0.05 ml portions were removed and frozen at –80° C until digested with the single-strand-specific nuclease, S₁, as described¹³. C_0t values (C_0 is the concentration of cellular DNA in moles of nucleotide per litre, and t is the time in seconds) were calculated as suggested by Britten and Kohne²⁸ as A_{260} per ml per 2 $\times$ h, and corrected to a monovalent cation concentration of 0.18 M²⁹. *a*, Annealing of ³H-DNA probe prepared from the xenotropic (S-tropic) BALB/c murine C-type virus¹⁴ grown in canine thymus cells (FCf2Th) to DNA extracted from the BALB/3T3 cell line (○), normal BALB/c liver (Δ), the canine thymus cell line (FCf2Th)¹⁷ infected with the S-tropic virus (●); and a rabbit corneal cell line (SIRC)¹⁴ infected with this virus (▲). Various concentrations of unique sequence rabbit cell line (SIRC) DNA (the highly reiterated DNA sequences that anneal by a C_0t of 50 were removed by fractionation on hydroxylapatite) and 4×10^4 acid precipitable c.p.m. of ³H-thymidine-labelled unique sequence SIRC DNA (0.5 μ g) were annealed (□) as described above. *b*, Annealing of ³H-DNA probe prepared from the baboon virus¹⁷ grown in the human rhabdomyosarcoma (A204) cell line to DNA extracted from liver (Δ) and lung (○) tissue of two normal baboons, and from the canine thymus cell line (FCf2Th) infected with baboon virus (●). Self-annealing of the canine cell line (FCf2Th) unique sequence DNA (□).

Table 1 Nucleic acid reassociation kinetics of viral ³H-DNA probes to the DNA of various tissues, cell lines, and cells infected with virus

Virus*	Tissue	$C_0t_{1/2}$ †	Cell line‡	$C_0t_{1/2}$	Cell line (infected)	$C_0t_{1/2}$
Murine (S-tropic)	Mouse liver	1.2×10^2	BALB/3T3	1.1×10^2	S-tropic/SIRC	1.5×10^3
Baboon (M7)	Baboon lung	1.0×10^2	S2CL3	1.2×10^2	S-tropic/FCf2Th	1.2×10^3
	liver	1.4×10^2	NT		M7/FCf2ThCL5	1.0×10^3
	spleen	1.4×10^2				
Feline (RD114)	Cat liver	1.8×10^2	CCC CL6	2.0×10^2	CCC/FCf2Th	1.5×10^3
	kidney	1.8×10^2	FFc60WF	1.9×10^2	RD114/M413	2.0×10^3
Rat (RT21C)	Rat liver	0.6×10^2	RT21C	1.2×10^2	NT§	
			LLC-WRC256	0.9×10^2		
Porcine (PK15)	Pig liver	1.5×10^2	PK(15)	1.5×10^2	NT§	

NT, not tested.

* ³H-DNA probes were prepared from the viruses as described in Fig. 1. S-tropic murine virus¹⁴ was grown in the canine thymus (FCf2Th) cell line. Baboon C-type virus (M7)¹⁷ was grown in the human rhabdomyosarcoma cell line (A204), and RD114 in RD cells¹⁵. Rat (RT21C)¹⁸ and pig (PK(15))⁸ viruses were spontaneously released from their respective cell lines.

† Half C_0t values represent the midpoint of the reannealing curves¹⁹. The reannealing rate of dog, baboon, human, cat, rabbit and mouse unique sequence cellular DNA has been shown¹³ to range between a half C_0t of 1.0×10^3 – 2.0×10^3 .

‡ The cells are described in the legends to Figs 1 and 2. S2CL3 is a BALB/c cell line spontaneously producing mouse C-type virus³¹, and LLC-WRC256 is a rat carcinoma line producing rat C-type virus³².

§ These viruses do not replicate to any extent in any mammalian cell line tested.

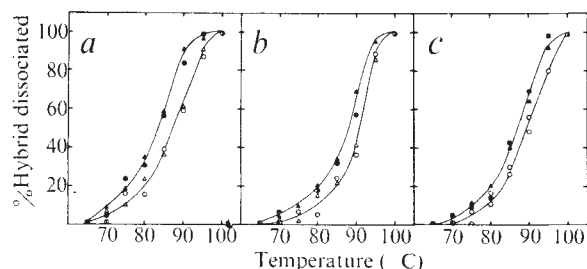


Fig. 2 Thermal stability of hybrids formed between viral ^3H -DNA probes and cellular DNA of various tissues and virus-infected cell lines. After hybridisation to a C_{0t} of 10^4 , aliquots (containing 600–1,200 c.p.m.) were heated for 5 min in 0.75 M NaCl at the indicated temperatures. The amount of hybrid remaining was determined with the single-strand-specific nuclease, S_1 . *a*, Thermal stability of hybrids formed between a ^3H -DNA probe prepared from the xenotropic (S-tropic) BALB/c virus¹⁴ grown in a canine thymus cell line (FCf2Th) and DNA extracted from the S-tropic virus-infected canine cell line (○); the virus-infected rabbit cell line (SIRC)¹⁴ (△); mouse liver (●); and a BALB/3T3 cell line (▲). *b*, The ^3H -DNA probe prepared from RD114 virus grown in RD cells¹⁵ was hybridised with DNA extracted from a canine thymus cell line infected with the endogenous cat virus, CCC (○); from a line of human embryonic fibroblasts (M413) infected with RD114 (△); from domestic cat liver (●); and from either CCC CL6 or FFC60WF feline cell lines³¹ (▲). *c*, The ^3H -DNA probe prepared from an endogenous baboon virus (M7) grown in the human rhabdomyosarcoma line A204¹⁷ was hybridised with DNA extracted from the canine thymus cell line infected with M7 (○); from baboon liver (●); and baboon lung (▲).

Table 1 summarises the half C_{0t} values obtained with ^3H -DNA probes prepared from several mammalian C-type viruses. A human or canine cell line infected with the endogenous domestic cat virus, RD114, contains only one copy in the cell DNA (half C_{0t} values 1.5×10^3 to 2.0×10^3), whereas normal feline tissues and cell lines contain multiple copies of genes related to the information in this virus (the half C_{0t} values range from 1.8×10^2 to 2.0×10^2). ^3H -DNA probes prepared from a porcine virus (PK(15)) and a rat virus (RT21C) were also hybridised, respectively, to the DNA of normal pig and rat. Again, multiple copies of these viral gene sequences are present in the DNA of their species of origin (half C_{0t} values are 0.6×10^2 to 1.5×10^2). Since neither of these viruses can replicate in the mammalian cell lines so far tested, we cannot determine the reiteration frequency of the viral genes in exogenously infected cells. Each of the five mammalian species examined thus contains multiple copies of their endogenous virogenes. In striking contrast is the situation where a cell is infected with and actively producing a heterologous C-type virus; only one to two copies of the virus information is present in the cellular DNA.

Labelled DNA probes prepared from mouse leukaemia viruses (Kirsten and AKR) grown in mouse cells have also revealed multiple copies (five to seven) in mouse cell DNA^{6,7}, as described here using probes from the xenotropic BALB/c virus grown in heterologous cells. Furthermore, the mouse mammary tumour viruses, B-type viruses, are also present in multiple copies in normal murine cell DNA²⁰. The results described here for the five mammalian systems may not apply to the subgroup E endogenous chicken virus, where fewer viral copies (one or two) have been reported in normal tissues⁵. Some mammalian cells transformed by avian sarcoma virus, however, acquire one viral copy per haploid genome⁴, as do the cells infected with heterologous mammalian C-type viruses.

To examine whether the multiple copies of C-type viruses in the cellular DNAs of these mammalian species represented identical copies of one set of virogenes or a family of related sets of virogenes, the thermal stability of the hybrids was determined. Base-pair mismatching results in the formation of hybrids that melt at a lower temperature—the effect of mismatched base pairs on the thermal stability is between 0.7°C and 1.6°C per 1% altered base pairs¹⁹. Figure 2 shows the melting curves obtained

when single-stranded ^3H -DNA transcripts of murine, RD114, and baboon C-type viral RNA were hybridised to the DNA from normal murine, cat and baboon tissues, respectively, and to the DNA of heterologous mammalian cells infected with these viruses. As Fig. 2*a–c* shows, the hybrid formed with DNA extracted from the species of origin of each virus melted $2–4^\circ\text{C}$ lower than the hybrid formed with the DNA of a heterologous infected cell line. This result did not depend on the cell line in which the virus was grown; for example, the thermal stability data obtained with DNA extracted from either the canine or human cell lines infected with the endogenous cat viruses (RD114/CCC group) was higher than that obtained with various normal cat cellular DNAs. Thus, the multiple copies of C-type viruses in these mammalian species (murine, feline and baboon) were not all identical in sequence to the endogenous viruses isolated from these species.

What is the evidence that the various endogenous C-type viruses isolated from each of these species are different? In the murine system, three endogenous C-type viruses have been isolated from the BALB/3T3 cell line that differ not only in their host range but also in some of their nucleic acid sequences^{14,21}. The C-type viruses from two rat cell lines (LLC-WRC256 and RT21C) share only a partial (40–60%) nucleic acid sequence homology (unpublished observations). We suggest, therefore, that the other mammalian species tested will also contain sets of virogenes which will be only partially related to the ones that have so far given rise to infectious C-type viruses.

The presence of multiple copies of viral information is a useful criterion with which to identify the species of origin of an endogenous mammalian C-type virus. A feline virus (RD114) satisfies this criterion, as do the mouse, baboon, rat and pig C-type viruses. Moreover, it is striking that the copy number clusters in a relatively narrow range (5 to 15 copies per haploid genome). Cells (including clonal isolates) and fresh tissues, to the extent that they have been examined, have comparable numbers of copies. These multiple copies of genetically transmitted C-type virus information may be present in the germ cells of each animal of a species (virogene hypothesis¹) or they may evolve by recombination and gene duplication during the lifetime of the animal (protovirus hypothesis²²). Taken together with experiments showing considerable expression both of viral RNA and viral specific proteins in embryos and various normal tissues^{23–26}, the results described here suggest that C-type genes have been preserved selectively during evolution and that their expression has some normal physiological function. Whether during their evolution as cellular genes the virogenes of a single species have diverged from one another in function, as they have in structure, remains to be determined.

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Scanning immunoelectron microscopy of mouse B and T lymphocytes

ALTHOUGH B and T lymphocytes (derived from the bone marrow or thymus, respectively) are functionally different, they are not morphologically distinguishable by ordinary light and transmission electron microscopy (TEM). Thus immunospecific labels had to be developed for these methods to identify populations of lymphocytes^{1,2}. Scanning electron microscopy (SEM) has been reported to show that T cells are smooth and B cells are villous^{3,4}. This conclusion, however, is controversial, and it is uncertain whether T and B lymphocytes can be distinguished by SEM appearance^{5,6}. We have now used immunospecific latex markers, which can be visualised by SEM, to identify cells with known T and B-associated antigenic surface determinants in the mouse. Mouse B cells have small stubby projections and are generally smoother than mouse T cells, which may be either villous or have few projections, but there is no distinct morphological difference.

An indirect latex labelling method, adapted from that of LoBuglio *et al.*⁷, was used to localise B-cell-associated and T-cell-dependent antigenic determinants on mouse lymphoid cells. The cells were treated with rabbit antisera to B or T-cell-associated antigens and then labelled with latex spheres coated with sheep anti-rabbit IgG (SARG). Rabbit anti-mouse Ig (B-cell-associated antigen, RAMB) was prepared by immuni-

sing rabbits with γ -globulins precipitated twice with ammonium sulphate and purified on DEAE. Rabbit anti-mouse brain (T-cell-associated antigen, RAMB) antiserum was prepared by immunising a rabbit with one brain equivalent in complete Freund's adjuvant (CFA) (Difco) in several subcutaneous sites, boosting after 1 month and collecting the serum after a further month. The antiserum was heat-inactivated at 56°C for 45 min and absorbed with one-third volume packed mouse red blood

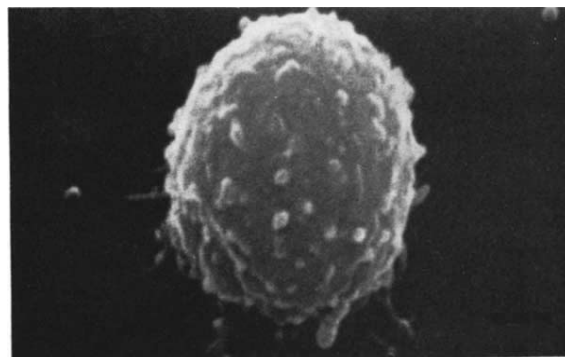


Fig. 1 Nu/Nu lymphocytes (B cells) sensitised with RAMB (T-cell-associated antigen). Cells were 5–7 μ m in diameter and relatively homogeneous with a smooth surface. Usually 10–20 basal pseudopodial attachments and 50–100 micro-stubs covering the remaining surface could be visualised after attachment to glass. If the cells were treated with RAMB and followed by SARG-latex (see text), no latex label was visualised on the cell surfaces.

cells. Functionally, this antiserum plus complement removed all (1) helper cell activity for primary and secondary immune responses in mice, (2) concanavalin A and phytohaemagglutinin mitogenic activity in mouse spleen populations and (3) cytotoxic effector cells, but had no detectable effect on plaque-forming cells, lipopolysaccharide mitogenic activity and precursor or memory B-cell populations as assessed by *in vitro* immune induction. SARG was prepared by immunising each of two sheep with purified H chains of rabbit IgG in CFA intramuscularly. Booster injections were given and the sheep were bled 3 and 7 weeks later. The antisera were specific for IgG by immunoelectrophoresis and double diffusion in agar. Latex particles nonspecifically adsorb γ globulins, and latex spheres with adsorbed γ globulin which has specific antibody activity will attach to the specific surface antigen⁷. One milligramme of

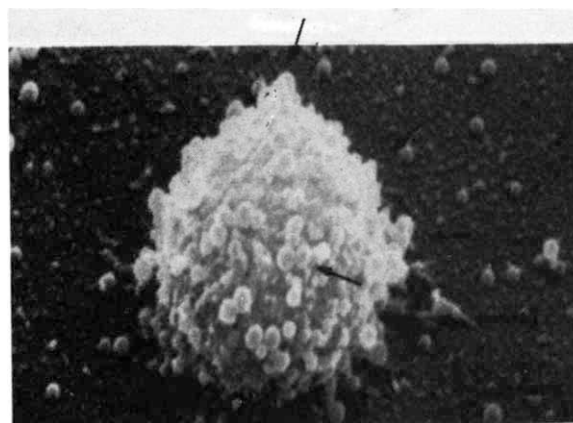


Fig. 2 Nu/Nu lymphocytes sensitised with RAMB (B-cell associated antigen). Eighty per cent of cells treated with RAMB and SARG-latex (see text) show specific surface labelling for surface immunoglobulin (arrows). Usually a heavy background of latex particles (on the glass) accompanied positive labelling due to possible shearing and removal of surface label during preparation for SEM.

latex spheres (0.2 μm diameter) (Monsanto) was coated with 75 μl of SARG (SARG-latex) for 30 min at room temperature before labelling.

Mouse lymphocytes were prepared from the mesenteric lymph nodes of either congenitally athymic, Nu/Nu, or BDF1 mice. The lymph nodes were teased apart, cell aggregates were allowed to settle and then the cell suspensions were washed three times in basal salt solution. Functionally, Nu/Nu cells are primarily a B cell population^{8,9}, and BDF1 lymph node cells are a mixture of B and T cells; enriched T-cell preparations were obtained from BDF1 lymph nodes by collecting the first 10 ml effluent fraction from a nylon wool column¹⁰.

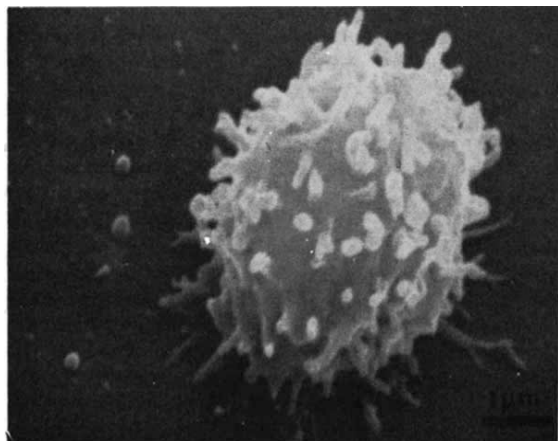


Fig. 3 BDF1 T cells sensitised with RAMG (B-cell-associated antigen). BDF1 lymphocytes (T cells) passed through the column showed heterogeneity in surface topographical characteristics. Usually 70–80% of the cells (5–7 μm in diameter) were villous, with 20–100 surface projections (50–800 nm long). The remaining 20–30% of the cells had smoother membranes, some without microvilli (not shown). If the cells were treated with RAMG and SARG-latex (see text), no surface labelling was seen.

Cell suspensions were prepared for latex labelling by settling $1\text{--}2 \times 10^6$ cells on to a 2 cm^2 circular glass coverslip for 30 min at 37° C. The cells were fixed to the glass in 1% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, for 30 min and rinsed twice in buffer. Sensitising or coating antiserum (RAMG or RAMB) was added for 30 min at room temperature and unreacted antiserum was rinsed off with PBS. Before addition of SARG-latex label, coverslips were rinsed in 0.2 M diphosphate, pH 9.2. SARG-latex was added for 30 min and unattached particles were washed off by flushing with 0.2 M diphosphate. The remaining antibody-ligated latex particles attached to the cell surface were stabilised by a second fixation in glutaraldehyde. γ Globulins (SARG) adsorbed on the latex particles may dissociate from the latex at low molarities and pH between 5.5 and 9.0, so that postfixation is needed to prevent dissociation of the latex from the cell surface. Even so, during SEM preparation the shearing forces on the large latex spheres may remove them from the cell surface, and we usually found many shed latex spheres with positive labelled cells. Labelled cells were dehydrated in a graded series of ethanol concentrations. The specimens were continued through Freon 113 and Freon 13 for critical point drying¹¹, coated with 400 Å of palladium and examined on an ETEC U-1 scanning electron microscope with an accelerating voltage of 30 kV and specimen tilt of 30° or 45°.

SEM of labelled B lymphocytes (Nu/Nu cells) showed that they had a relatively smooth surface membrane, with prominent, stub-like digitations, but no distinct finger-like microvilli (Figs 1 and 2). If B-cell preparations were sensitised (coated) with normal rabbit serum or RAMB antiserum followed by SARG-latex, no label was detectable on the surface (Fig. 1). However, surface immunoglobulin determinants could be visualised on 80% of the lymphocytes with SARG-coated latex if the cells were sensitised with RAMG (Fig. 2). Except

for the presence of label, there was no detectable difference between labelled and unlabelled cells. B lymphocytes labelled for surface Ig had 50–150 densely distributed latex spheres attached to the surface. With specific labelling there were sometimes many free latex particles on the surrounding glass, probably due to shearing as above. The dense pattern of Ig labelling observed was different from the patchy and sparse distribution seen on rabbit peripheral blood lymphocytes (PBLs) which have quantitatively less surface Ig¹². The unlabelled population of Ig-negative cells (20%) probably represented a null population or at least cells with insufficient surface Ig to be detected by this labelling technique.

Lymphocyte preparations enriched for T cells (eluted from nylon columns) were heterogeneous in surface topography. Between 70 and 80% were villous (Figs 3 and 4); the remaining 20–30% had smoother surfaces, some with few or no microvilli. Latex label could not be detected on T-cell-enriched preparations sensitised with normal rabbit serum or RAMG followed by SARG-latex (Fig. 3). Specific cell surface labelling, however, was visible on more than 90% of cells sensitised with RAMB (Fig. 4). The label was usually seen as distinct patches (1–2 μm across) containing 5–15 spheres. Most villous cells had quantitatively more surface label than smoother cells. Because differences in morphology may be due to cell preparations, for example, elution off a nylon wool column may induce formation microvilli in a subpopulation of T cells, we examined normal unfractionated BDF1 lymph node cells. Of these 30–40% were labelled with RAMB and SARG-latex. The morphology of the labelled cells was similar to that of the T-positive cells (90%) in the T-enriched population (that is, most of the labelled cells were villous), suggesting that nylon column fractionation did not affect significantly the surface architecture of the passed cells. The observed differences in T-cell morphology and labelling may represent different functional populations, especially since the more villous cell types had more T-specific surface label.

Cell preparation and processing for SEM may affect the surfaces of mouse lymphocytes. Rabbit PBLs are smooth in suspension by SEM and TEM, but develop microvilli and pseudopodia when adherent to glass for SEM¹³. In the study reported here all cells were allowed to adhere to glass for 30 min to produce conditions for villous formation. Environmental factors, including temperature¹⁴, cell preparation¹⁵ and cell cycle¹⁶ can affect the topology of the surface membrane. Thus these parameters must be evaluated before the SEM appearance of individual cells can be used for identification.

The controversy of 'hairy' B cells and 'smooth' T cells is unresolved⁶. Contrary to our findings, with mouse lymphocytes,

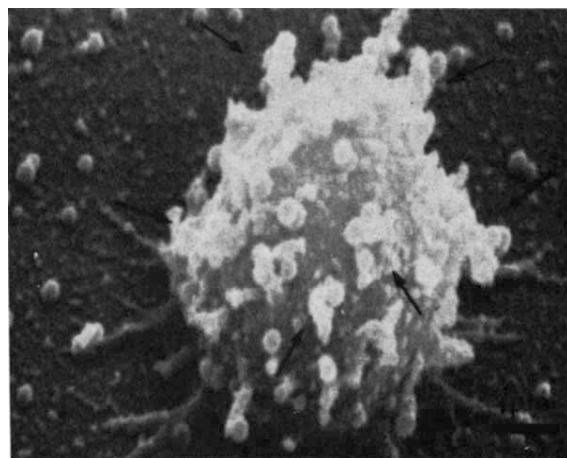


Fig. 4 BDF1 T cells sensitised with RAMB (T-cell-associated antigen). T lymphocytes treated with RAMB and SARG-latex (see text) showed specific surface labelling of brain associated (T-dependent) antigen. Latex label was visualised on 90% of the cells as distinct patches (arrows).

SEM observations of human lymphocytes showed that B cells usually had numerous microvillous projections, whereas T cells were smooth with relatively few microvilli^{3,4}. In our experiments mouse B cells were relatively smooth, while most T cells had extensive microvilli and pseudopodia. We interpret these morphological distinctions with caution because there is usually a great deal of heterogeneity in surface architecture among a given population of cells. Even in our enriched population of T cells, a wide variety of positive labelled stubby and villous cells was found. Thus, we conclude that B or T cell identification by SEM is tenuous without immunospecific markers.

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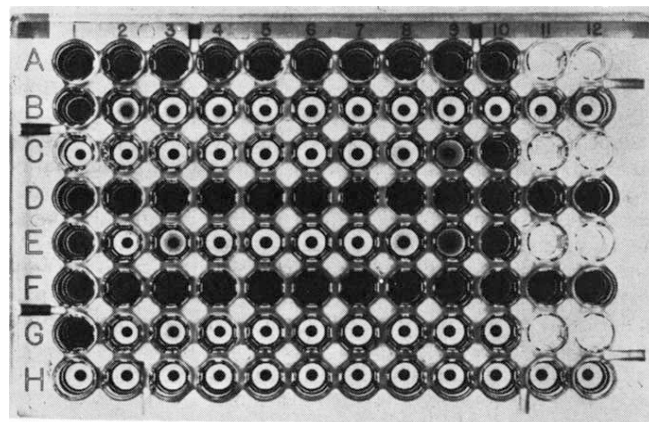


Fig. 1 Agglutination of type O, trypsinised human erythrocytes (1% suspensions in Microtiter wells) by soybean agglutinin and by N-acetyl-D-galactosamine and D-galactose. Four titrations of soybean agglutinin as serial twofold dilutions in PBS or PBS plus monosaccharide are shown. The soybean agglutinin concentration in the first well of each titration series (A-1, C-1, E-1, G-1) was $1,000 \mu\text{g ml}^{-1}$. The twenty-first and twenty-second wells of each titration (B-11 and B-12, D-11 and D-12, F-11 and F-12, H-11 and H-12) were controls and contained no soybean agglutinin. In the first titration (A-1 through B-10), no monosaccharide was present. The wells of the second (C-1 through D-10), third (E-1 through F-10) and fourth (G-1 through H-10) titrations of soybean agglutinin and the wells of their respective controls contained 0.01 M N-acetyl-D-galactosamine (Pfanstiehl, lot no. 8076A), 0.01 M D-galactose (Pfanstiehl, lot no. 9145), and 0.01 M D-galactose (Baker, lot no. 2-0411), respectively.

four times with PBS. Haemagglutination assays were performed as described previously² in unperturbed wells of Microtiter plates (Cooke Engineering Co.) with 1% (v/v) erythrocyte suspensions and twofold serial dilutions in PBS of the agglutinin of interest. The sedimentation patterns of the 1% erythrocyte suspensions were read after 2 h at room temperature. A positive pattern, indicating agglutination, is uniform effacement of the bottom of the well by erythrocytes; a negative pattern, indicating no agglutination, is a circular clump of erythrocytes surrounded by a concentric, clear zone (Fig. 1).

Our initial observation was that some preparations of inhibitory sugars produced positive haemagglutination patterns in Microtiter wells with concentrations of soybean agglutinin insufficient for agglutination (Fig. 1). As expected, soybean agglutinin-mediated haemagglutination was inhibited by N-acetyl-D-galactosamine and by two preparations of D-galactose. It was unexpected that two of the three inhibitory monosaccharides tested N-acetyl-D-galactosamine (Pfanstiehl, lot no. 8076A) and D-galactose (Pfanstiehl, lot no. 9145), caused haemagglutination (wells C-10 through D-12 and E-10 through F-12, respectively) when the concentration of soybean agglutinin was too low for haemagglutination. No agglutination was produced by agglutinating preparations of N-acetyl-D-galactosamine or D-galactose in the presence of high concentrations of soybean agglutinin (wells C-1 through C-8 and E-2 through E-8). Therefore, it seems that some interaction between soybean agglutinin and the agglutinating compounds present in the monosaccharide preparations prevented haemagglutination by the agglutinating compounds. The nature of this interaction is not understood; the haemagglutinating material may bind to soybean agglutinin. The interaction is obviously not inhibited by the parent monosaccharide solution. A similar phenomenon was observed with D-mannose and concanavalin A (Miles-Yeda).

Preparations of these and other monosaccharides were surveyed for haemagglutinating activity in the absence of lectin (Table 1). Since not all preparations of D-galactose and N-acetyl-D-galactosamine caused haemagglutination, we conclude that the haemagglutinating activity is not due to the

Haemagglutinins in commercial preparations of monosaccharides

We have found that several commercial preparations of monosaccharides possess haemagglutinating activity. This activity was observed with monosaccharide concentrations close to those usually used to inhibit the biological effects of lectins. The activity is not due to the monosaccharides themselves but is associated with a high molecular weight compound(s) active in some preparations of monosaccharides.

Soybean agglutinin was purified by affinity chromatography as described previously¹. Human erythrocytes, drawn into EDTA and used within 7 d, were washed four times with phosphate buffered saline (PBS), pH 7.2-7.4, trypsinised as 4% (v/v) suspensions in PBS with 0.01% (w/v) crystalline trypsin (Sigma, type XI) for 90 min at 37° C and washed again

monosaccharides but must be produced by another compound active in some preparations.

We have investigated the nature of the haemagglutinating compound present in D-galactose (Pfanstiehl, lot no. 9145). Trypsinised erythrocytes were about ten times more susceptible to agglutination than were non-trypsinised erythrocytes; types A, B and O erythrocytes were equally agglutinable. Agglutination was not totally reversed by nine washes of the agglutinated erythrocytes with 100-fold volumes of PBS. Haemagglutinating activity was removed from a 0.2 M galactose solution by pre-incubation for 2 h at room temperature with 5×10^7 type O erythrocytes per ml. The haemagglutinating activity in the sugar solution was destroyed by boiling for 90 min; the activity was resistant to up to ten cycles of freezing and thawing.

The haemagglutinating activity in galactose (Pfanstiehl, lot no. 9145) was found in the retentate and could be concentrated quantitatively and washed free of monosaccharide by ultrafiltration over an XM-50 ultrafilter (Amicon) which nominally retains proteins of molecular weight greater than 50,000. The activity was nearly completely retained by an XM-300 ultrafilter that retains proteins of molecular weight greater than 300,000. The haemagglutinating activity in solutions of D-mannose (Baker, lot no. 1-9855) and D-glucose (Mallinckrodt, lot no. XDX) could be separated in a similar fashion.

Preliminary characterisation of the concentrated compound, washed free of D-galactose by ultrafiltration indicated that the compound had a high carbohydrate content by the phenol-sulphuric acid assay³. A Lowry determination⁴ indicated a protein content equivalent to 90 µg of bovine serum albumin per mg of dried material, but the carbohydrate content may have interfered with the procedure⁵; preliminary analysis revealed only trace amounts of amino acids. Ultraviolet spectra of agglutinating preparations of unfiltered D-galactose (~ 0.2 M) show a peak at 280 nm (~ 0.25 absorbance units). The material responsible for the absorbance could be removed partially by ultrafiltration, however, without demonstrable loss of haemagglutinating activity. Possible sources of these high molecular weight haemagglutinating compounds are the

biological source of the sugar (for example, ivory-nut meal for mannose⁶), biological agents used during production of the sugar (for example, bakers' yeast for galactose⁷), and polymerised products of sugars and/or sugar derivatives⁸ formed during manufacture and purification of the sugar. Heat-polymerised furfurals⁸ do not appear to be responsible for the haemagglutinating activity as atmospheric refluxing of aqueous solutions of galactose and glucose sufficient to produce intense ultraviolet absorption at 280 nm did not produce haemagglutinating activity. We conclude tentatively that the haemagglutinating material obtained from galactose is a heat labile macromolecule composed primarily of carbohydrate with, perhaps, a minor protein component.

The presence in many monosaccharide preparations of a large molecular weight compound with haemagglutinating activity may have important consequences for several kinds of studies on the biological effects of monosaccharides, and for studies of any biological system containing or utilising monosaccharides. Such considerations are of obvious importance in tissue culture where glucose is a component of culture media and in studies involving the effects of monosaccharides on tissue culture cells and intact organisms as, for example, in sugar transport⁹. The presence of these compounds in monosaccharides may affect sugar-dependent characterisation of the physical properties and biological specificities of lectins and even the purification of lectins by affinity techniques utilising monosaccharides. Until the properties of these high molecular weight compounds are more fully understood, ultrafiltration can be used for complete removal of the high molecular weight compounds and the associated haemagglutinating activity from the monosaccharides.

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Table 1 Survey of sugar preparations for haemagglutinating activity

(a) Preparations with haemagglutinating activity

	Lowest haemagglutinating concentration (mM)
N-acetyl-D-galactosamine (Pfanstiehl 8076A)	4
D-galactose (Pfanstiehl 9145)	4
D-galactose (P-L Biochemical 331261)	8
Galactose (Pfanstiehl 9638)	8
D-mannose (P-L Biochemical 332651)	8
D-mannose (Calbiochem 34148)	16
D-mannose (Baker 1-9855)	16
D-mannose (Pfanstiehl 10012)	32
2-deoxy-D-glucose (Pfanstiehl 9466)	40
D-glucose (Mallinckrodt XDX)	160
D-fucose (Pfanstiehl 8742)	160

(b) Preparations without haemagglutinating
Activity

Lactose (Pfanstiehl 9471)
N-acetyl-D-galactosamine (Pfanstiehl 9918)
D-galactosamine hydrochloride (Pfanstiehl 9003)
D-galactose (Baker 2-4011)
Galactose (Fisher 793901)
N-acetyl-D-glucosamine (Pfanstiehl 9245)
Methyl-α-D-mannopyranoside (Pfanstiehl 9375 and 9589)
D-arabinose (Pfanstiehl 9288ML)
L-fucose (Pfanstiehl 9698ML)
Sucrose (Baker 30388)

Haemagglutinating activity was assayed in Microtiter plates with a 1% suspension of trypsinised, type A erythrocytes and serial twofold dilutions of the monosaccharides in PBS as described in the text. The lowest millimolar concentrations of monosaccharides producing haemagglutination are shown. The highest concentration tested was 160 mM. The sources and lot numbers are indicated in parentheses.

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Molecular and crystal structure of deoxyguanosine 5'-phosphate

RECENT crystallographic studies of the dinucleosides ApU (ref. 1) and GpC (ref. 2) have given experimental proof for the base pairing arrangement proposed by Watson and Crick for the DNA double helix³. Another striking feature of this structure relates to the torsional angle about the C5'-C4' bond in the phosphate-sugar backbone chain. In the Crick and

Watson model⁴, this conformation is *gauche-trans* (GT). Crystal structures of 5'-nucleotides, dinucleosides and dinucleotides so far studied, however, have shown only the *gauche-gauche* (GG) conformation about this bond. The GG conformer is also the only one found in the refined models of the proposed structure of the double helical nucleic acids and polynucleotides⁵⁻⁷. The only nucleotide with a GT conformation is 6-azauridine-5'-phosphate⁸ which is not a normal monomer unit of nucleic acids. It is also reported that 5'-dGMP assumes preferentially GT conformation in solution⁹.

We wish to report here the molecular structure of the DNA monomer unit, deoxyguanyldic acid (dGMP-5') as determined by a complete three-dimensional X-ray structure analysis. The nucleotide shows the GT conformation about the C-5'-C-4' bond similar to that occurring in the Crick and Watson model.

Crystals of the disodium salt of deoxyguanosine 5'-phosphate were grown by slow diffusion of alcohol into aqueous solutions of the compound¹⁰. Thin platy crystals about 2 mm long were obtained in about 3 week. The crystal belongs to monoclinic system with space group $P2_1$. The unit cell dimensions are $a = 16.00 \text{ \AA}$; $b = 10.73 \text{ \AA}$; $c = 5.575 \text{ \AA}$; $\beta = 101.9^\circ$; $d_{\text{obs}} = 1.64 \text{ g cm}^{-3}$; $d_{\text{cal}} = 1.64 \text{ g cm}^{-3}$; $Z = 2$. Density measurements carried out using carbon tetrachloride-bromoforn mixtures indicated the presence of four water molecules in the asymmetric unit. Intensity data were collected by multiple film, equiinclination, Weissenberg technique for h01-h91 and hk0 layers using $\text{CuK}\alpha$ radiation. The data were measured visually by comparison with precalibrated graded intensity scale. A three-dimensional Patterson function was computed and the phosphorous atom was located from the Harker section. The structure was solved by symbolic addition

$\phi_{\text{O}5'-\text{O}1'} = 62.5^\circ$ and $\phi_{\text{O}5'-\text{C}3'} = 174.8^\circ$. As mentioned earlier this is the first structure to show this conformation which is close to that found in Watson-Crick DNA model ($\phi_{\text{oo}} = 38^\circ$, $\phi_{\text{oc}} = 155^\circ$).

In model building studies and refinement on nucleic acid structures one tends to use the structural parameters and torsional angles within the constraints prescribed by the results of single crystal X-ray studies on nucleic acid constituents. It seems to us that the conformational features observed in dGMP-5' have also to be considered in possible polynucleotide conformations and foldings particularly of the DNA. The GT conformer and the O1' *endo* puckering of sugar ring are also of direct relevance to the concept of rigid nucleotide unit¹⁵.

We thank Professor V. S. Venkatasubramanian for his interest and Dr K. Venkatesan and Mr S. Ramakumar for providing the modified version of the 'Mutan' program. T.P.S. thanks the UGC authorities for financial support.

Note added in proof:- An accurate independent X-ray analysis of this nucleotide has now been reported by Young, Tollin and Wilson^{16,17}. Our findings are in close agreement with the conformational features reported by them.

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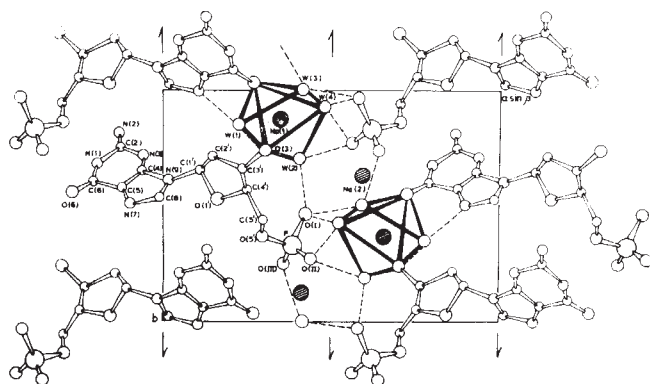


Fig. 1 Structure of dGMP-5' $\text{Na}_2 \cdot 4\text{H}_2\text{O}$ viewed down c axis. One of the sodium atoms ($\text{Na}(1)$) has an octahedral coordination shown by thick lines. Hydrogen bonds are indicated by broken lines. The stacking pattern of the bases belonging to molecules related by a ' c ' cell translation is not shown for the sake of clarity.

method. Phases for 290 reflections were obtained using the program 'Mutan'¹¹. An E map was computed for the best set which gave a Karle R factor for 28.59%. The entire molecule with two sodium ions and one water oxygen atom were identified from the E map from a knowledge of the position of phosphorous atom and by model building approach. The current structure has an R factor of 9.0%. The view of the structure down c axis is given in Fig. 1.

The glycosidic torsional angle χ_{CN} is 52.6° . The relative orientation of the base with respect to the sugar is therefore, *anti*. The χ_{CN} value is within the range expected of β -purine nucleotides. The best four atom plane for the deoxyribose moiety passes through $\text{C}1'-\text{C}2'-\text{C}3'-\text{C}4'$. The $\text{O}1'$ atom shows an *endo* puckering, being displaced by $0.6 \text{ \AA}$ from this plane in striking contrast with the other three DNA monomers where the furanose ring is puckered either with $\text{C}2'$ or $\text{C}3'$ (refs 12-14).

The conformation about the $\text{C}5'-\text{C}4'$ bond is GT with

Errata

In the article "Volatilisation of mercury and organomercurials determined by inducible R-factor systems in enteric bacteria" by J. Schottel *et al.* (*Nature*, **251**, 335; 1974), in the last two lines of the legend to Fig. 1 and also in the penultimate line of para. 3, J53(471a) should read J53(R471a) on each occasion.

In the article "Subunit structure of chromatin" by M. Noll (*Nature*, **251**, 249; 1974) line 7 of the legend to Fig. 4 should read ' $\dots$ and $\bar{V}_m = 9.2 \text{ ml}$ in $0.2 \text{ mM} \dots$ ' and not as originally printed.

matters arising

Do molecular biologists come of age in Aries?

WINDSOR has concluded that, "More molecular biologists were born under the sign of Aries than any other sign. More taxonomists were born under the sign of Cancer than any other sign and relatively few were born under Scorpio." Lest acceptance of these findings induces prospective parents to time the birth of their offspring to maximise their chances of becoming molecular or taxonomic biologists, a modest statistical analysis of Windsor's data seems in order.

Table 1 contains the frequencies, arranged by Sun sign and biological bent, of the birthdates of 812 biologists in the sample observed by Windsor¹. The relative frequencies of births for the general population of the United States for 1934 by Sun sign were estimated by adding the products of the monthly relative frequencies by the respective fraction of each of the two months contributing to a given Sun sign interval as given by Parker and Parker².

In the χ^2 tests for goodness of fit³ to the data in Table 1, the critical value is $\chi^2_{0.05, 11} = 19.675$ if $P \leq 0.05$ is the significance level to reject the null hypothesis, H_0 : that each sample has the same frequencies as the general population. Since $0.75 \leq P(\chi^2 \geq 7.282) \leq 0.90$ for the taxonomists, $0.10 \leq P(\chi^2 \geq 16.368) \leq 0.25$ for the molecular biologists, and $0.25 \leq P(\chi^2 \geq 11.580) \leq 0.50$ for the combined sample of 812 biologists, the null hypotheses cannot be rejected. That the samples of taxonomists and molecular biologists could have come from the same population is confirmed by the heterogeneity χ^2 test: $0.25 \leq P(\chi^2 \geq 12.070) \leq 0.50$.

As might be expected, the same conclusion that the biological discipline of the 812 scientists is independent of the Sun sign of birth is borne out by a 2×12 contingency table^{3,4} which involves no assumptions about the birth frequencies per Sun sign for the general population. In this case, $0.25 \leq P(\chi^2_{11} \geq 11.495) \leq 0.50$.

Even if the χ^2 tests had permitted the rejection of the null hypotheses, the validity of Windsor's conclusions would have been restricted to biologists in *American men and women of science*⁵ whose birthdays were listed. Other factors such as age and sex proportions among taxonomists, molecular biologists, and the general population would have complicated analysis and interpretation even further.

Table 1 Frequencies (f) of birthdates by Sun sign from a sample of 812 biologists¹ and expected frequencies (F) if the proportions were the same as in the general population (see text)

Sun sign	General population %	Taxonomists		Molecular biologists		Cumulative	
		f	F	f	F	f	F
Aquarius	9.05	26	30.98	35	42.57	61	73.55
Pisces	8.18	31	28.00	36	38.48	67	66.48
Aries	8.11	28	27.76	58	38.15	86	65.91
Taurus	8.46	30	28.96	32	39.80	62	68.76
Gemini	8.08	31	27.66	39	38.01	70	65.67
Cancer	8.84	38	30.26	41	41.59	79	71.85
Leo	8.67	32	29.68	32	40.79	64	70.46
Virgo	8.98	31	30.74	42	42.24	73	72.98
Libra	8.17	25	27.97	41	38.43	66	66.40
Scorpio	7.98	18	27.32	41	37.54	59	64.86
Sagittarius	7.75	27	26.53	40	36.46	67	62.99
Capricorn	7.64	25	26.15	33	35.94	58	62.09
Total	99.91	342	342.00	470	470.00	812	812.00
χ^2			7.282		16.368		11.580

While I hope that this modest analysis will be instructive and entertaining to students of biostatistics, I regret that astrologers may be disappointed, although they usually stress² that birth place and planets' positions are the determining factors of the birth chart or 'horoscope' used to suggest the likelihood of certain events.

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DR WINDSOR REPLIES—Dr Colás certainly provides an appropriate criticism of my Sun sign findings.

The statistical method I used, before publication, was a non-parametric analysis of a manifold population as described by Tate and Clelland¹. Considering the 12 Sun signs as a manifold population, the hypothesis tested was that the distribution of birthdays was uniform, $H_0 = A_r = T = G \dots = P = 1/12$. $\chi^2 = [(f^0 - f^e)^2]/f^e$, where f^0 is the frequency of characters in the sample and f^e is the frequency expected under the null hypothesis, that is, the average.

$$\chi^2 = [(A_r - f^e)^2]/f^e + [(T - f^e)^2]/f^e + [(G - f^e)^2]/f^e \dots + [(P - f^e)^2]/f^e.$$

χ^2 for molecular biologists = 13.818; χ^2 for taxonomists = 8.666. At 11 degrees of freedom the probabilities of the hypothe-

ses being true are 0.025 and 0.600, respectively. Since the hypothesis is rejected in both cases, therefore, the distributions were not random.

I compared molecular biologists with themselves and I compared taxonomists with themselves. I did not compare them with each other or with the general population. Molecular biologists and taxonomists are simply different kinds of biologists and must be considered independently or else the distinction is lost. Comparing them with the general population is unnecessary because it is obvious that, for the most part, they did indeed come from the same general population. The problem is not to identify similarities and establish origins, but to separate components and identify their characteristics. I used *American men and women of science* as my data base because it is a readily accessible, reproducible sample. Its validity can only be determined by gathering additional data. To cite one instance of possible invalidity, many practising molecular biologists are probably not listed as such, but appear classified as biochemists or physiologists, since the scientists describe their own disciplines.

My data were, admittedly, meagre, but it was hoped that their publication would inspire, or even goad, scientists with more resources to pursue this line of research by acquiring and analysing larger data bases. The basic concept of extraterrestrial influences on human activities is so important that it certainly deserves much more investigation than is now being performed.

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¹Tate, M. W., and Clelland, R. C., *Non-parametric and shortcut statistics*, 51-53 (Interstate, Danville, Illinois, 1957).

Ascorbic acid and nitrosamines

EDGAR¹ has proposed that ascorbic acid might inhibit the carcinogenic action of nitrosamines and other carcinogens that may act by alkylation. The rationale was that ascorbate might be alkylated *in vivo* before the carcinogens can react with cell macromolecules. The experimental basis for Edgar's thesis was the statement by Kamm *et al.*² that ascorbate inhibited the liver necrosis induced by dimethylnitrosamine (DMN). This statement, however, was presented without experimental details and was subsequently withdrawn³.

The main concern of the study by Kamm *et al.* was the inhibition by ascorbate of the liver toxicity induced by oral administration of aminopyrine plus nitrite. This study followed our report in 1972 suggesting, on the basis of *in vitro* experiments, that ascorbate might be used to block *in vivo* formation of N-nitroso compounds from nitrosatable chemicals (for example, drugs), since ascorbate efficiently reduces nitrite⁴. Subsequently, the report by Kamm *et al.*⁴ appeared. Greenblatt⁵ found similar results in mice to those of Kamm *et al.*, but stated that ascorbate did not affect DMN toxicity. We found that ascorbate prevented liver damage from gavage of dimethylamine plus nitrite to rats and, from experiments presented in detail, that ascorbate did not significantly affect the production by DMN of liver necrosis and elevated serum transaminase levels⁶. Ascorbate did not affect transplacental carcinogenesis in rats by ethylnitrosourea, but inhibited carcinogenesis by ethylurea plus nitrite⁷.

We are concerned that our original suggestion should not be extended without basis to the hypothesis that ascorbate might have a much wider inhibitory action on carcinogens. The interesting suggestion of Edgar is not supported by the results reviewed here, which were mostly made public after Edgar's paper was submitted.

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● *Nature* regrets that when this article was first published (**250**, 684; 1974) it contained certain typographical errors which may have caused confusion.

Scrapie

IN a group of Herdwick sheep genetically selected for susceptibility to subcutaneous injection with SSBP/1 scrapie brain pool, Pattison reported¹ the occurrence of two cases in uninjected animals. He concluded "... that these two cases arose spontaneously by genetic selection". The simplest explanation of these two cases, however, is that they were due to infection with scrapie from some unrecognised source with later cases resulting from lateral and maternal transmission of the infection.

The factual parts of Pattison's report are in accord with our own findings. Cheviot sheep have been selected successfully for increased or decreased susceptibility to scrapie^{2,3} following subcutaneous injection with the same source of agent as that used by Pattison. These Cheviot sheep were bred on a geographically isolated farm, away from known cases of scrapie and for the first 7 yr no natural cases of scrapie occurred. Since 1968, however, when a natural 2-yr-old case occurred in the positive selection line, the incidence of natural cases has built up as shown in Table 1, and 81 of the 83 cases have occurred in the positive line. The other two cases have occurred recently in a group of 11 positive-line × negative-line crosses born in 1970. As in Pattison's cases these natural Cheviot cases are

easy to distinguish on histological criteria. Because many different strains of scrapie agent can be recognised by the characteristic type, severity and distribution of brain lesions which they produce⁴, the histological difference found in the sheep is direct evidence that no component of SSBP/1 is involved in the natural Cheviot cases and this is supported by a failure so far to isolate from them either 22A, 22L or 22C (three strains of agent known to be present in the SSBP/1 pool⁵).

The simplest explanation for the occurrence of the natural Cheviot and Herdwick cases is that the necessary conditions for the total 'isolation' of a flock from direct or indirect contact with scrapie agent are not yet understood. This is not surprising in view of the well known extreme resistance of scrapie agents to physical or chemical inactivation. Also, Icelandic evidence supports the view that there can either be long term persistence of scrapie infectivity on farms in the absence of sheep or that some vector is involved (ref. 6 and P. A. Palsson and B. Sigurdsson, unpublished). Quite apart from these considerations scrapie is known, quite definitely, to be naturally transmitted both laterally and maternally in field conditions (refs 7 and 8 and J. L. Hourigan *et al.*, unpublished) and among housed sheep⁹⁻¹².

One comment is fundamental: it should not be assumed that the Herdwicks and Cheviots which are susceptible to peripheral injection with SSBP/1 scrapie will be susceptible to all strains of scrapie or that the resistant lines will be resistant to all strains. Such an assumption is at the basis of Pattison's interpretation. The situation is clearly illustrated by work with scrapie in mice where it is not possible to describe one genotype as susceptible and another as resistant unless the strain of scrapie agent, dose of agent and route of infection are specified¹³.

Although the work with the Cheviot sheep is less advanced than that in mice, there is evidence that a similar situation applies in both species. Pattison cites his own report of scrapie infectivity being produced from apparently normal mouse tissue: these results were interpreted by him as evidence for spontaneous generation of scrapie infectivity ('unmasking')

Table 1 Incidence (%) of natural scrapie in two lines of Cheviot sheep selected for susceptibility (positive line) or resistance (negative line) to subcutaneous injection with SSBP/1 scrapie brain pool

Year born	1957-60	1961-65	1966	1967	1968	1969	1970
Foundation stock	0	—	—	—	—	—	—
Selection line { positive	—	0	1	7	37	23	39
negative	—	0	0	0	0	0	0

The flock size varied from 120-200 ewes and there were approximately equal numbers in the two selection lines.

¹ Edgar, J. A., *Nature*, **248**, 136-137 (1974).

² Kamm, J. J., Dashman, T., Conney, A. H., and Burns, J. J., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 747-749 (1973).

³ Kamm, J. J., Dashman, T., Conney, A. H., and Burns, J. J., in *N-Nitroso Compounds in the Environment* (edit. by Bogovski, P., Walker, E. A., and Davis, W.) (International Agency Research Against Cancer, Lyon, in the press).

but by others as being more likely to be due to accidental contamination¹⁴. If contamination is the correct explanation then the 'spontaneous' agent should be identical with one of the small number of strains in use in Pattison's laboratory but material has not been available for agent identification. Pattison also cites the work and interpretations of Parry¹⁵ but fails to quote the critical reanalysis¹⁶ of Parry's data which produced entirely different conclusions.

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Ageing cell cultures

Is there an irreversible loss of mitotic ability in mammalian cell populations during ageing¹? The results of continuous labelling alone can neither confirm nor refute this hypothesis.

Macieira-Coelho² continuously labelled cultures of human embryonic fibroblasts with tritiated thymidine. He found that the proportion of unlabelled cells declined to zero in a convex fashion. His result is an inevitable consequence of the continuous labelling method.

Macieira-Coelho did not distinguish between unlabelled dividing cells and unlabelled non-dividing cells. By definition non-dividing cells will not take up label and will not reproduce. If the initial proportion of unlabelled non-dividing cells is $q/2^0$, then after k population doublings this proportion will be reduced to $q/2^k$. The proportion of unlabelled dividing cells will decline at a faster rate since the number of dividing cells that are unlabelled also will decrease. The proportion of unlabelled cells is the sum of these two proportions. It will decline to zero in a convex fashion (Table 1).

It will decline whether time is measured chronologically, in population doublings, in split ratios or in cell generations³. It will decline whether the population is growing or static, for in a homeostatic population, where cell death is balanced by cell birth, the number of unlabelled cells will decline. It will decline whether or not un-

labelled cells continue to enter the division cycle.

The results reported by Macieira-Coelho² are artifacts of the continuous labelling method.

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¹ Good, P. I., and Watson, D., *Exp. Geront.*, **8**, 147 (1973).

² Macieira-Coelho, A., *Nature*, **248**, 421 (1974).

³ Good, P. I., *Cell Tissue Kinet.*, **5**, 319 (1972).

⁴ Smith, J., and Hayflick, L., *J. Cell Biol.*, **62**, 48 (1974).

DR MACIEIRA-COELHO REPLIES—I think the theoretical speculations made by Dr Good are more likely to be artifacts than the experimental results on which I have based my conclusions.

Dr Good says that the decline in the population of unlabelled cells is an inevitable consequence of the continuous labelling method. As a matter of fact, my data show very clearly that this is not the case, and that the proportion of unlabelled cells depends on the size of the inoculum. With high inocula not all the cells have time to enter the cycle before division stops due to cell crowding. Furthermore, if after a continuous labelling one finds only 6% of unlabelled cells, regardless of how this equilibrium is reached at resting phase (loss of non-dividers, dilution, and so on) it means that there are only 6% of cells which did not enter the S period. The possibility remains that these cells are slowly cycling cells delayed in other periods, a hypothesis most mathematicians do not like to consider.

Eight years ago I published results¹ which led to the conclusion that during the growth decline of human fibroblasts the cell population becomes strongly heterogeneous and the cells show a spectrum between the two extremes, that is, complete inhibition of division and the normal division cycle. What has to be kept in mind is the presence of a spectrum, and the experimental evidence suggests that there is no predominant cell type (that is, cells with fast, intermediate or long generation times, or non-dividers). If there is a fraction of cells which do

not divide at all in the population, this is a small fraction as my data have now shown. There is no reason whatsoever to make speculations about growth kinetics stating that this small fraction would play a bigger role than the rest of the spectrum of cells with a whole range of generation times.

¹ Macieira-Coelho, A., Ponten, J., and Philipson, L., *Expl Cell Res.*, **42**, 673 (1966).

Intelligence and handedness

GIBSON¹ has stated that his data do not support Levy's² contention that left-handed subjects have significantly lower visuospatial IQ's. Cohen's³ factorial study of the Weschler adult intelligence scale suggests that a more appropriate test of verbal IQ than the full Weschler scales used by both Gibson and Levy would consist of the average of age-weighted scores on subtests weighting on verbal comprehension (information, comprehension, similarities, vocabulary) and a test of visuospatial IQ would consist of subtests weighting on perceptual organisation (block design, object assembly).

Table 1 Comparison of two measures

		Right handed	Left handed
Verbal IQ	mean	123.3	125.5
	s. d.	5.36	7.08
Performance IQ	mean	114.6	112.7
	s. d.	6.82	6.72
Verbal comprehension score	mean	14.23	14.78
	s. d.	1.37	1.27
Perceptual organisation score	mean	12.37	12.4
	s. d.	1.60	3.54

Comparing a small sample of left- and right-handed subjects, ten of each group, no significant differences between the two groups were found on either the full verbal and performance IQ's or on the verbal comprehension and perceptual organisation scores (Table 1). These data support Gibson's findings.

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¹ Gibson, J. B., *Nature*, **243**, 482 (1973).

² Levy, J., *Nature*, **224**, 614 (1969).

³ Cohen, J., *J. Consult. Psychol.*, **21**, 451 (1957).

DR GIBSON REPLIES—A reanalysis of my data along the lines suggested by Roberts has given similar results. There are no significant differences between left-handed and right-handed subjects on either the verbal comprehension or perceptual organisation scores.

University of Cambridge

Table 1 Percentage of unlabelled cells at confluence, by type, following continuous exposure to labelled tritiated thymidine

Split Ratio	Observed ²	Theoretical	
	%	Non-dividers	Dividers
No transfer	100	%	%
1:1	79	16 (ref. 4)	84 (ref. 4)
1:2	25	16	63
1:4	3	8	17
		4	1

No correction was made in this table for cell death or cell loss during transfer³.